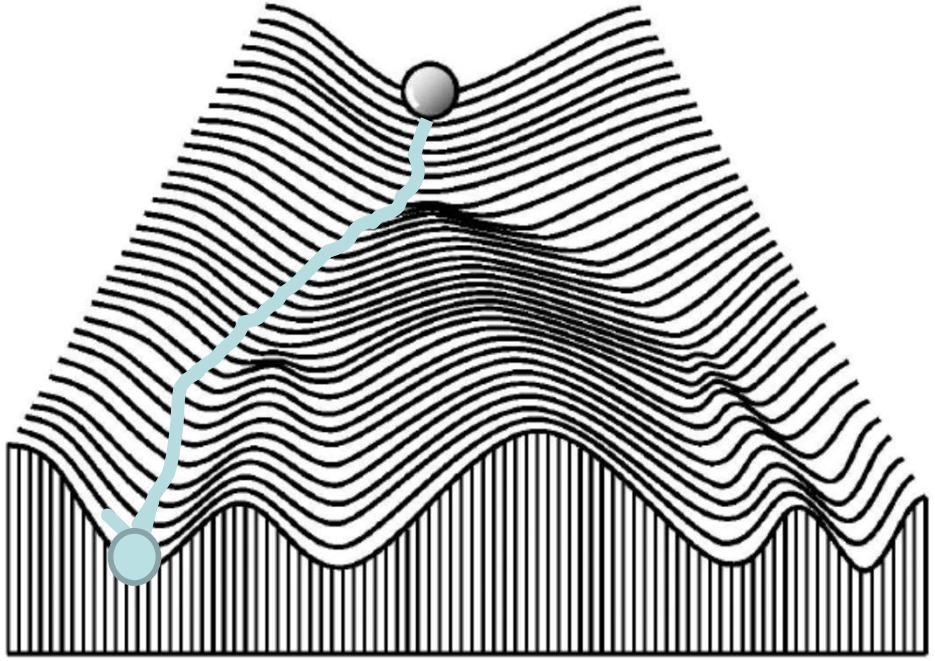
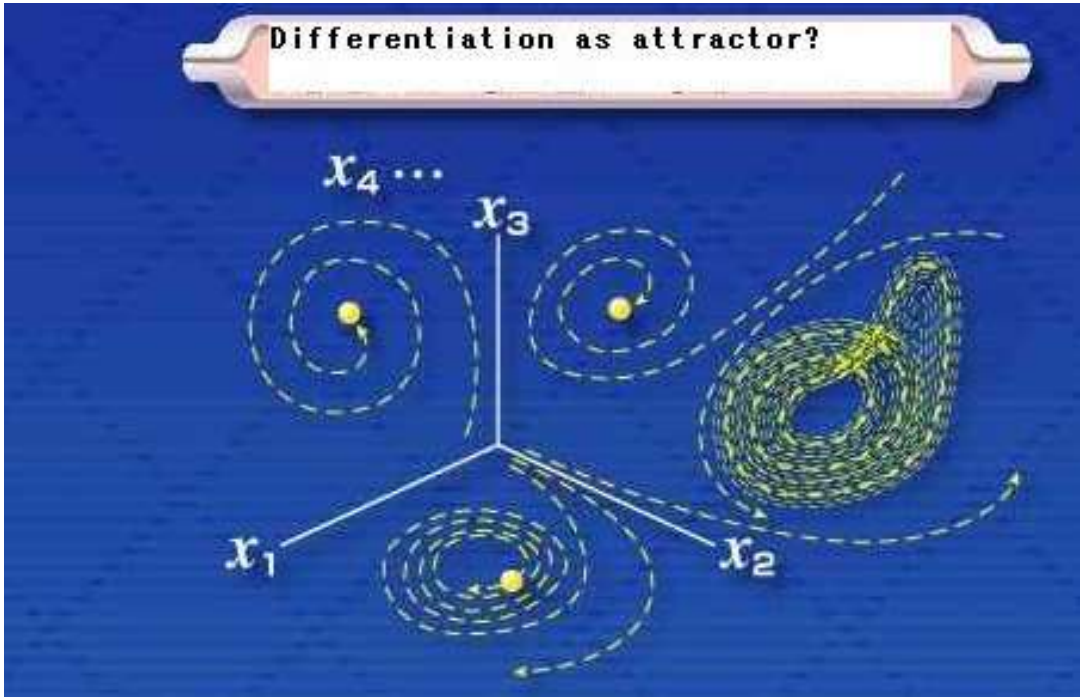
Cell Differentiation

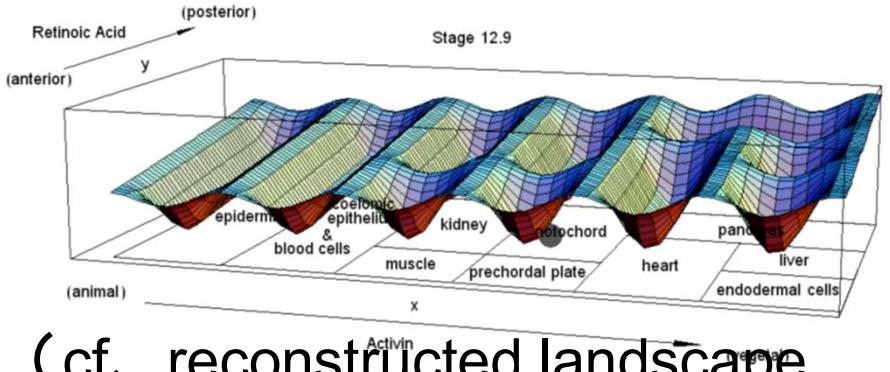
Waddington's Image





Cell types as Different attractors

Waddington's Canalization
(stability of each cell type)1958
How genes guide this process?



(cf、reconstructed landscape
From experimental data,
KK,Sato, with Asashima's group)

Dynamical System's View?
(attractor) (cf Kauffman mid60's)

Waddington, The strategy of genes, 1957

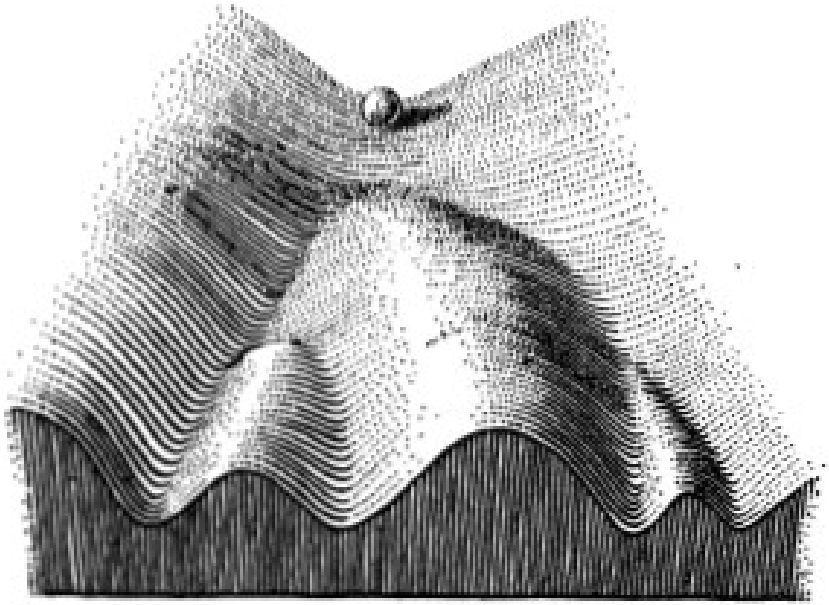


FIGURE 4

Part of an Epigenetic Landscape. The path followed by the ball, as it rolls down towards the spectator, corresponds to the developmental history of a particular part of the egg. There is first an alternative, towards the right or the left. Along the former path, a second alternative is offered; along the path to the left, the main channel continues leftwards, but there is an alternative path which, however, can only be reached over a threshold.

Epgienetic Landscape

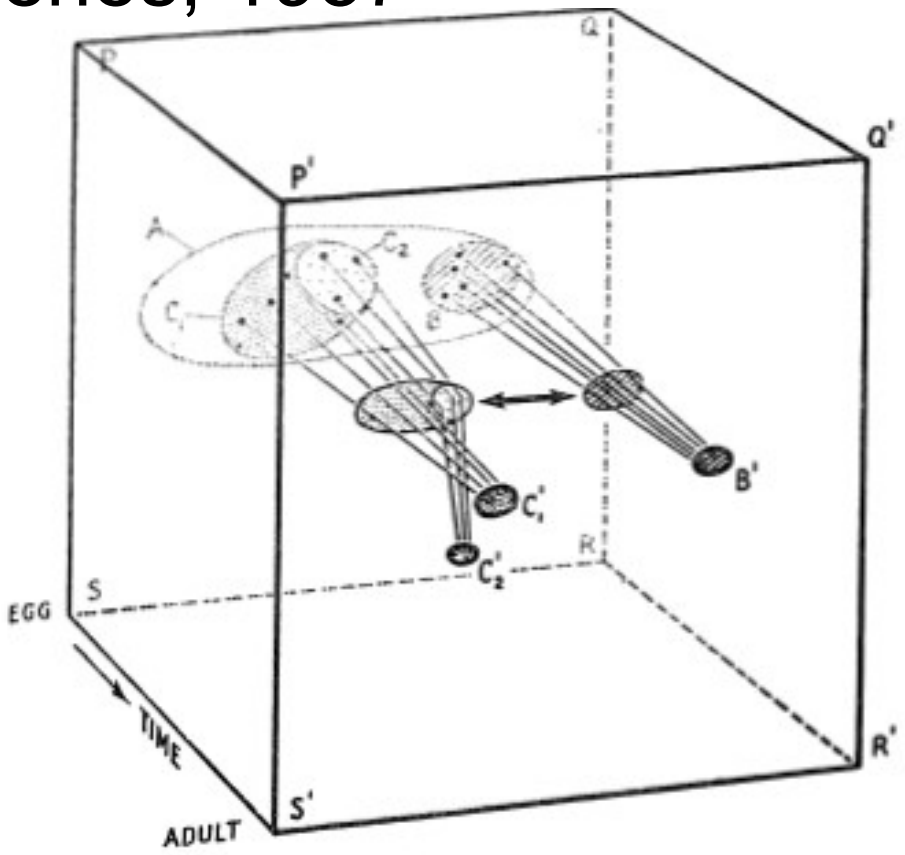


FIGURE 3

A phase-space diagram of development. The time axis runs perpendicular to the paper, from the plane $PQRS$ at the time of fertilisation to $P'Q'R'S'$ in the adult. The other two dimensions represent the composition of the system. The composition of the various parts of the egg (which in this case varies continuously, in a 'gradient' manner) originally lie within the area A. One region of the egg, with composition B, develops along a series of trajectories which converge towards the adult tissue B'. Another region, with composition $C_1 + C_2$ also begins to develop along a converging set of trajectories. At some stage during development, physical contact (heavy double arrow) occurs between the B region and C_2; a reaction occurs by which the C_2 trajectories are diverted so as to converge on C'_2; this represents an induction.

A phase-space diagram of development

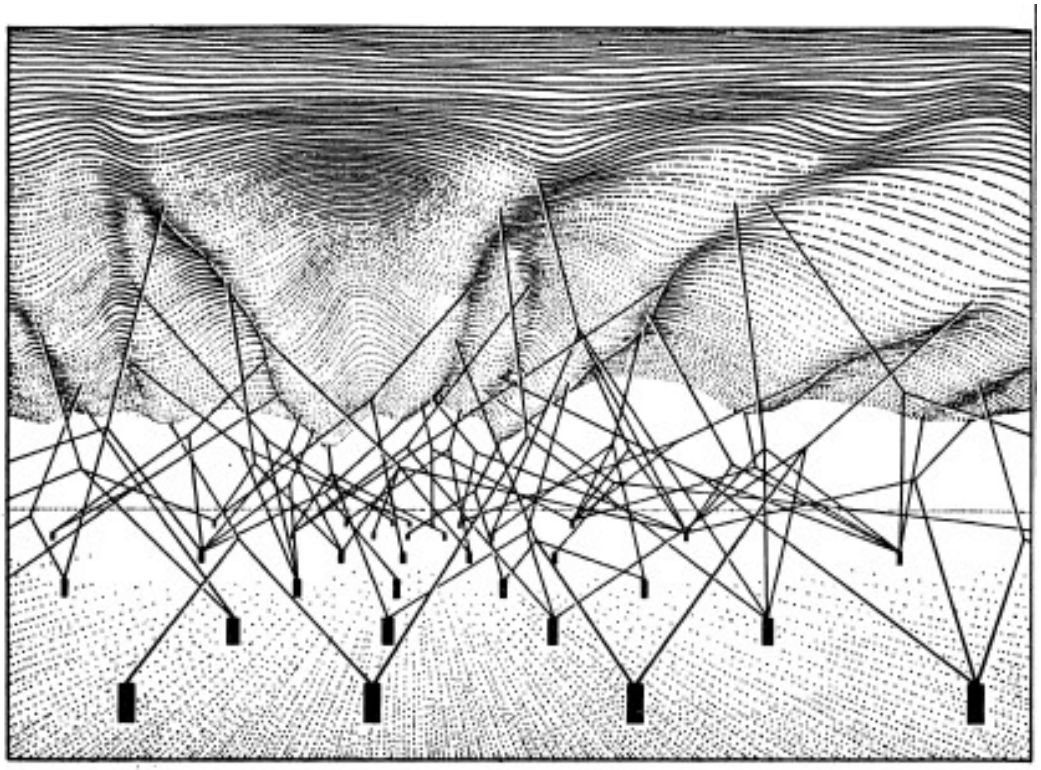
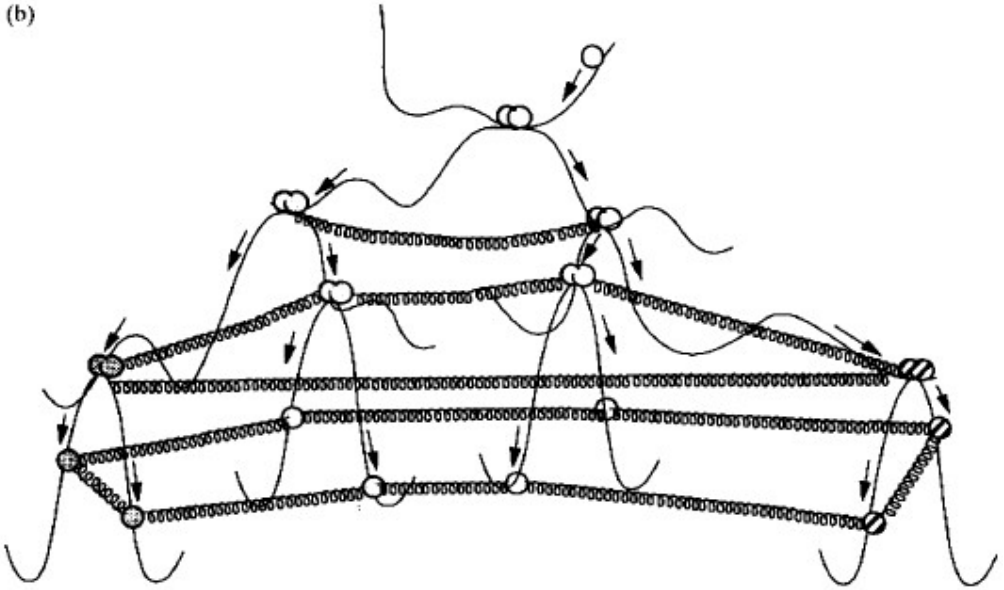


FIGURE 5

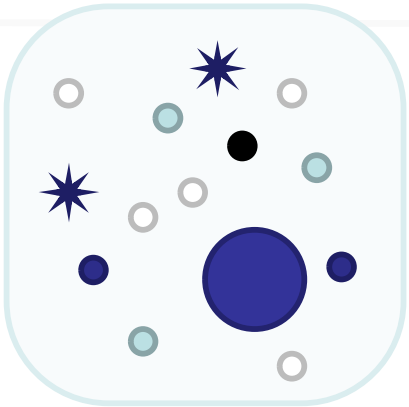
The complex system of interactions underlying the epigenetic landscape. The pegs in the ground represent genes; the strings leading from them the chemical tendencies which the genes produce. The modelling of the epigenetic landscape, which slopes down from above one's head towards the distance, is controlled by the pull of these numerous guy-ropes which are ultimately anchored to the genes.



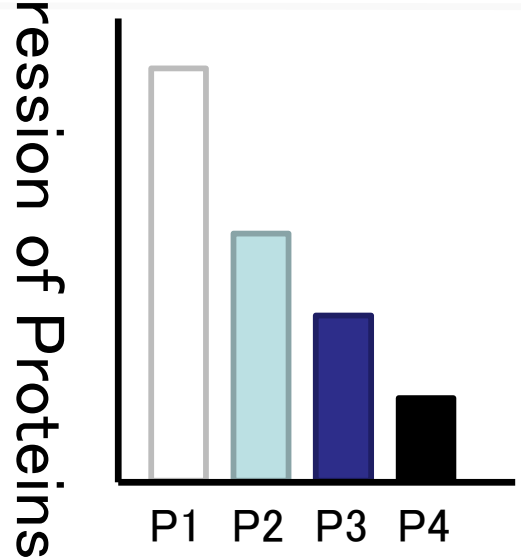
Kaneko,Yomo 97

The complex system of interaction underlying the epigenetic landscape

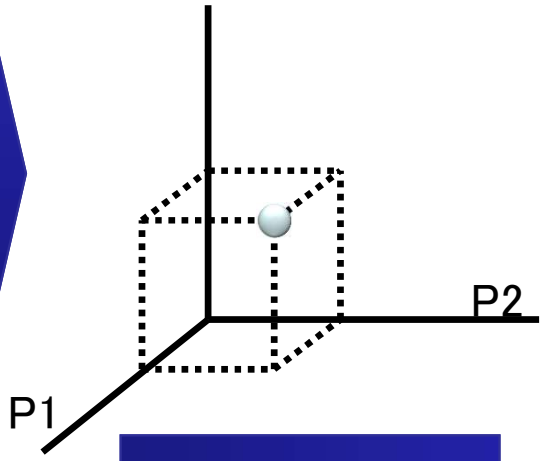
Dynamical Systems's View

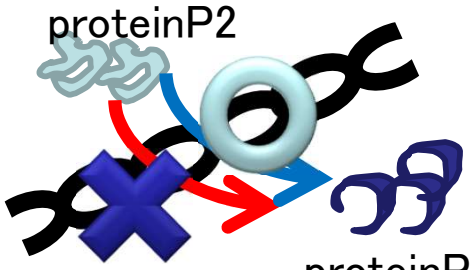


Cellular State



Cellular state as a point in N-dimensional space





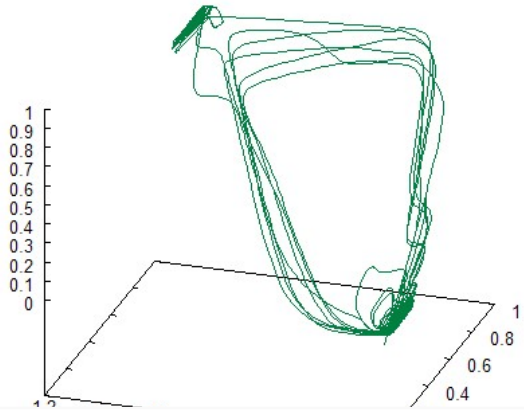
Mutual Activation /Inhibition

$$\begin{cases} \dfrac{dp_i^k}{dt} = m_i^k - p_i^k + D_i\left(\overline{p_i^k} - p_i^k\right) \\ \dfrac{dm_i^k}{dt} = \gamma\left(f_i\left(\{p_j^k\}\right) - m_i^k\right) \end{cases}$$

$$f_i(x) = g_i\left(\sum_j J_{ij}x_j\right)$$

$$g_i(x) = \frac{1}{1 + \alpha e^{-\beta(x+c_i)}}$$

Representation by differential eq.



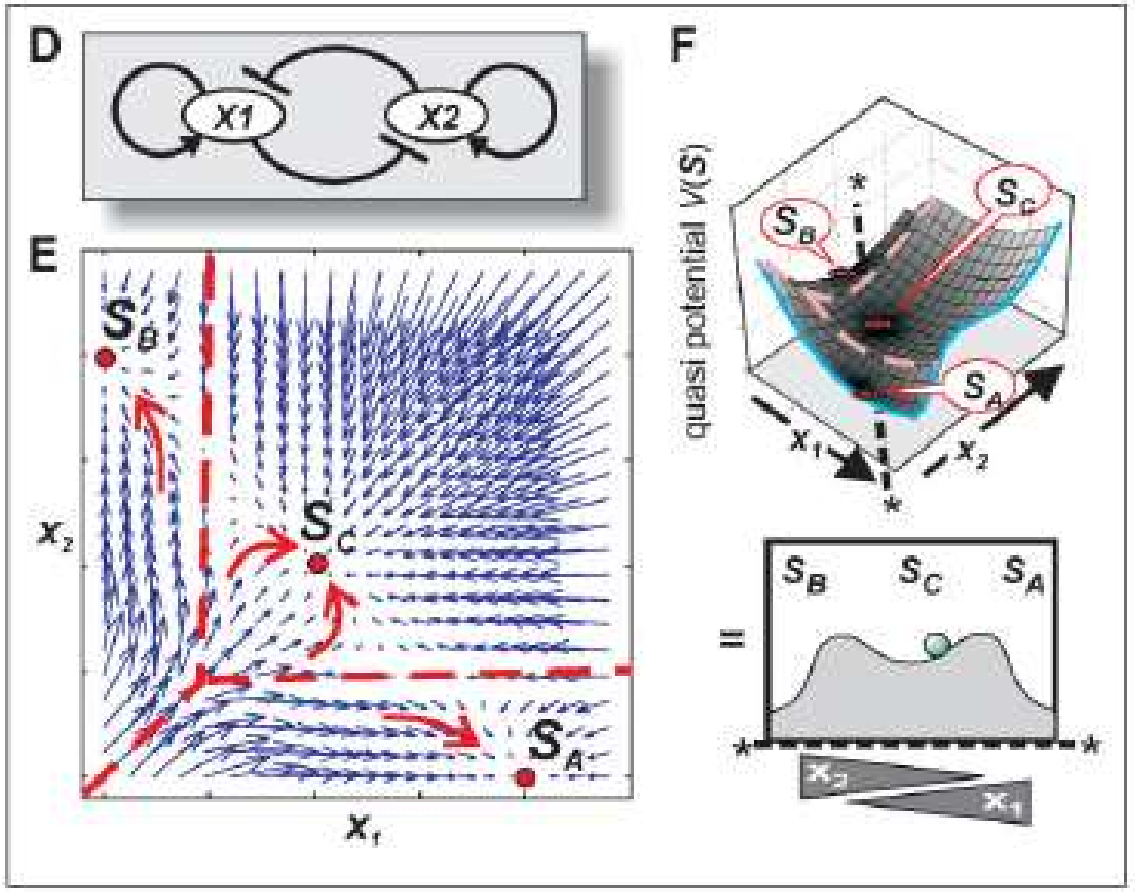
Temporal Evolution= Orbit

Problems in 'multiple attractor' view of 1-cell dynamics

How initial conditions for multiple attractors are chosen?

Just by noise?

too random?



Sui Huang Bioessay 2008

? Stability of differentiation process;Homeorhesis

?Stability of cell number ratio; ensemble level

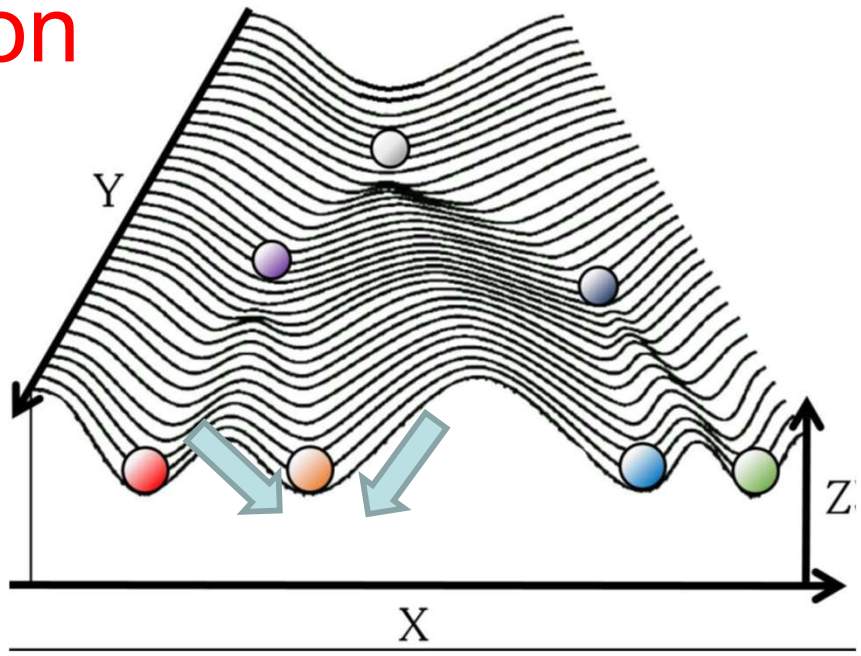
?Irreversible Loss of Pluripotency

(embryonic stem cell can produce any types, and…)

('time's arrow'?)

Relevance of cell-cell interaction?

←Each valley along X-direction attractor (→homeostasis)



But….. Landscape changes along Y-axis !?

'Homeorhesis' Y-direction

(rhesis , to flow →similar flow) Waddington p32

robustness in path.. Developmental process itself, i.e, landscape itself

but, what this landscape means?

Epigenetic Landscape and Homeorhesis???

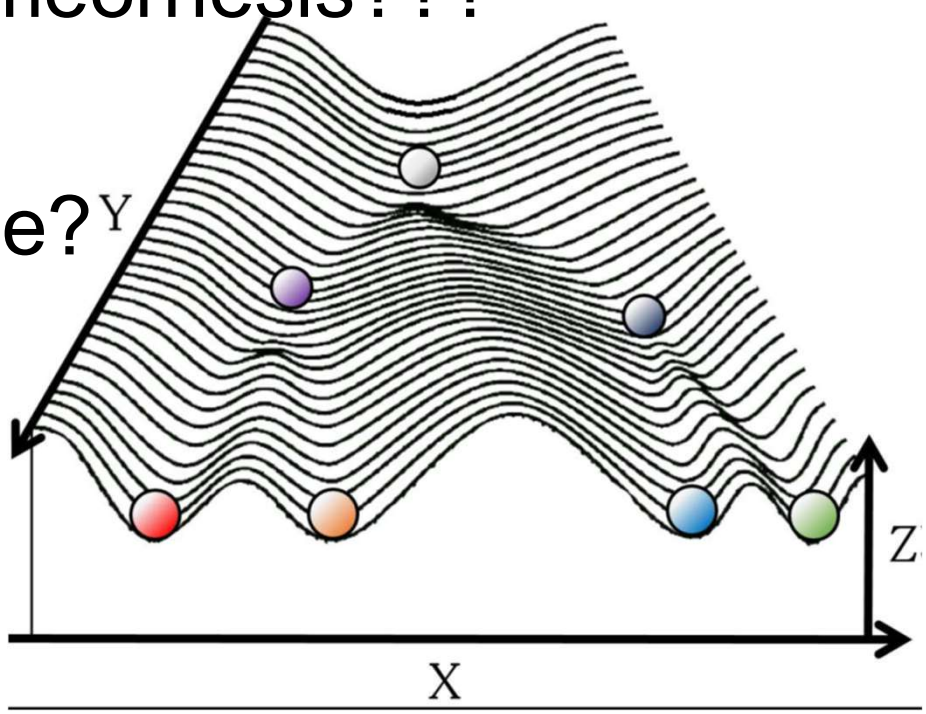
1) X,Y,Z axis ?

X: collective expression variable?

Z: plasticity (changeability)?

inverse of P(X)), cell # ratio

Y: slow change in gene expression dynamics?



2) Y developmental time vs time for X?

--Attraction into valley (attractor) in state- space :Fast

-- What is the Slow change in Y direction(depth)?

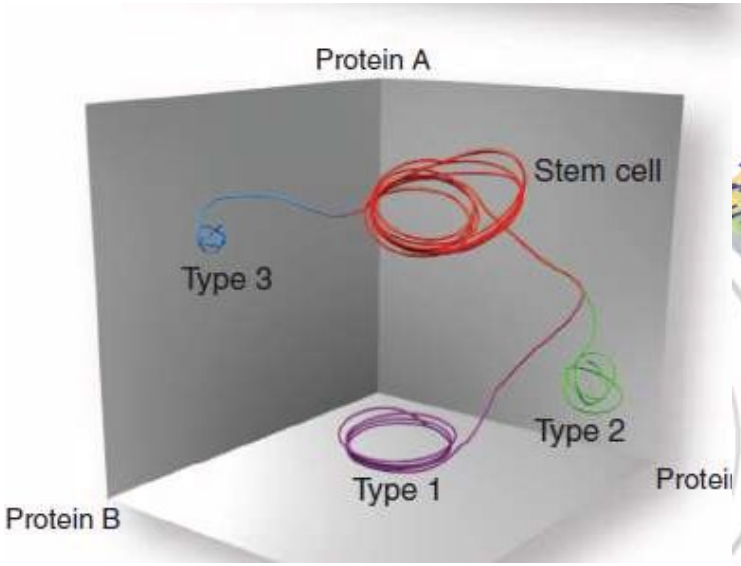
a)Slow developmental change (e.g.,in cell number)

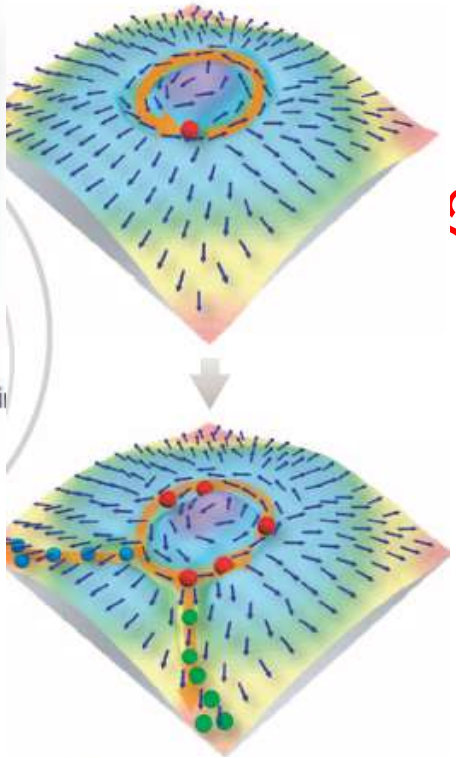
→ and according cell-cell interaction

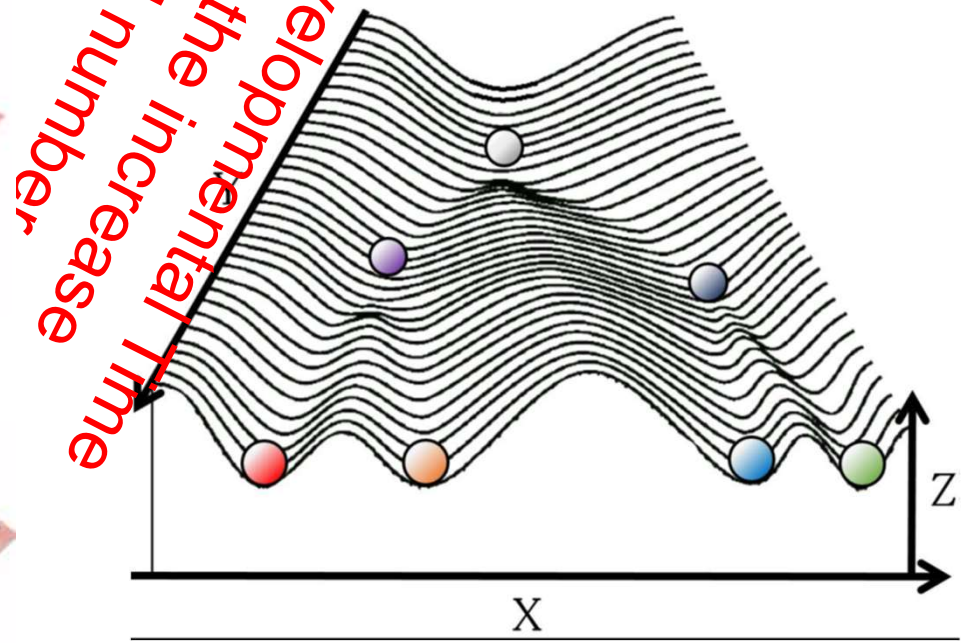
b)Fast expression + Slow epigenetic(?) change?

*Possibility（1）:

Y-axis: increase in cell number →cell-cell interaction (KK,Yomo1997,Furusawa-KK 1998,2001,…)







Science

A Dynamical-Systems View of Stem Cell Biology
Chikara Furusawa and Kunihiko Kaneko
Science **338**, 215 (2012);

Original: Furusawa, Kaneko 1999,2001

Possibility（2）

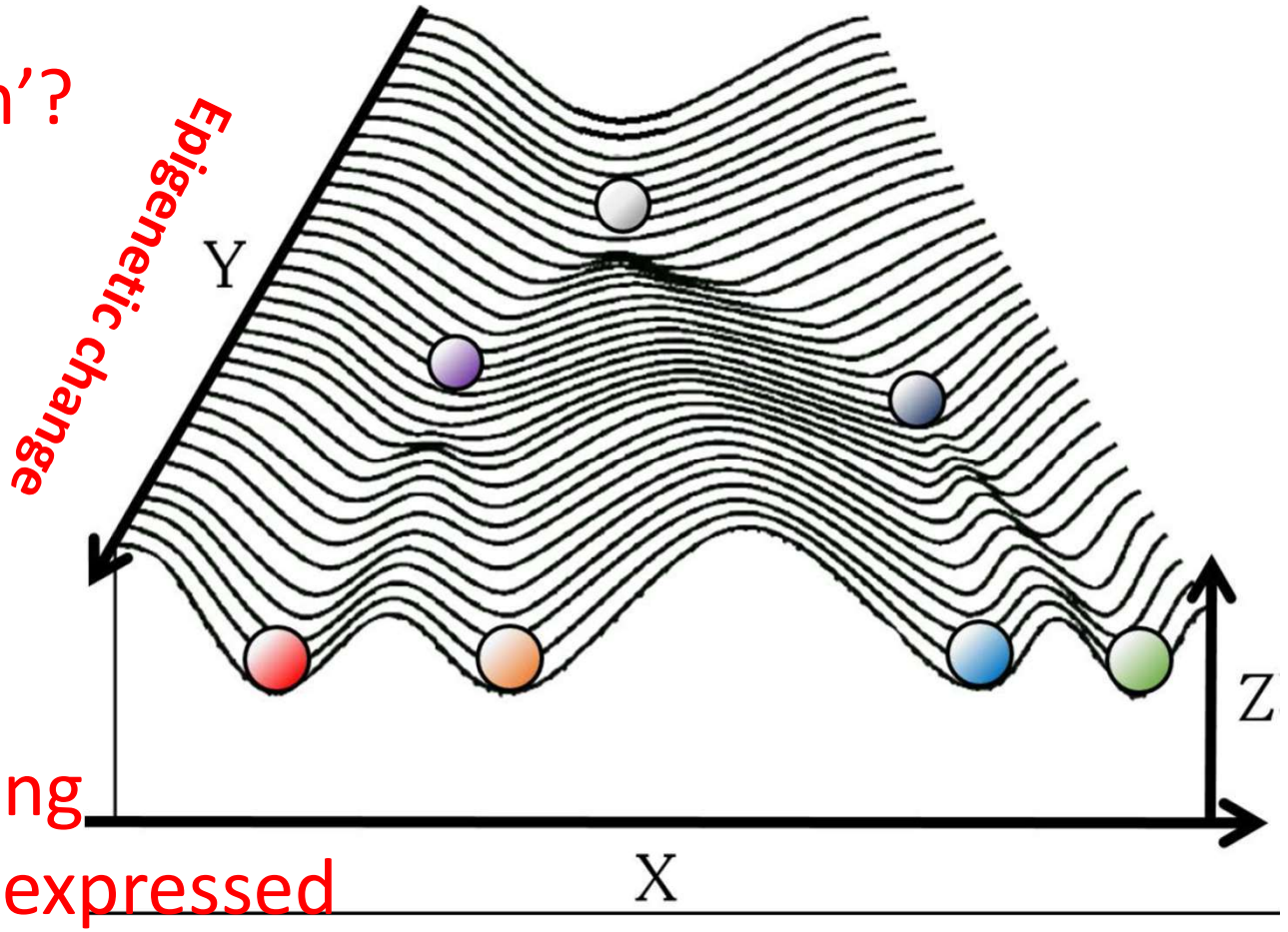
Y as the slow change in epigenetic modification?

(Matsushita, KK,2020, Phys Rev Res)

‘Epigenetic-Modification’?

←Histon Modification
Methylation,,,,

“Coarse-grained” model
→
Slow variable representing feasibility that a gene is expressed (by chromatin change)



Inter-Intra dynamics

Catalytic reaction dynamics

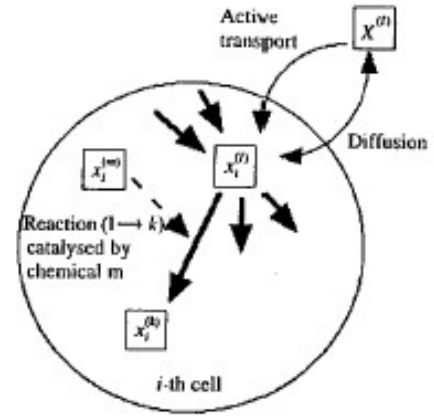
$$\frac{dx^m}{dt} = f_m(x^1, x^2, .., x^k)$$

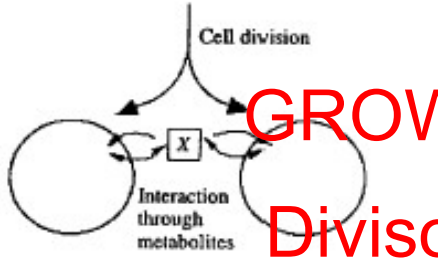
→ Growth of a cell

→Cell division

→ Cell-cell interaction

→ Differentiation??





GROWTH

Divisoin

FIG. 1. Schematic representation of our model. See the appendix for the specific equation of each process.

(In the division, state Xi is transferred (with small noise))

Coupled Dynamical Systems with growth in dimension

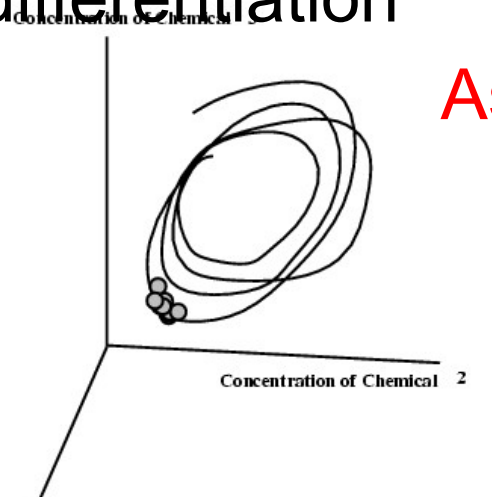
(all-to-all diffusion coupling (no spatial pattern)

→ irreversible and robust developmental process?

←based on the study of coupled dynamical systems

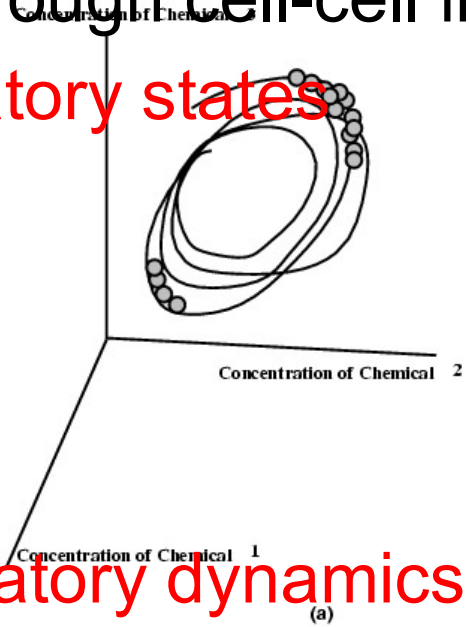
(cf, KK 84-90's)

synchronous division:
no differentiation

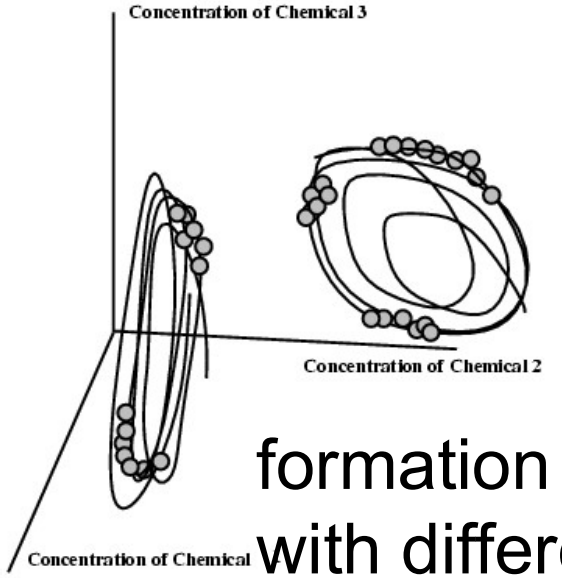


Instability of homogeneous state
through cell-cell interaction

Assuming oscillatory states

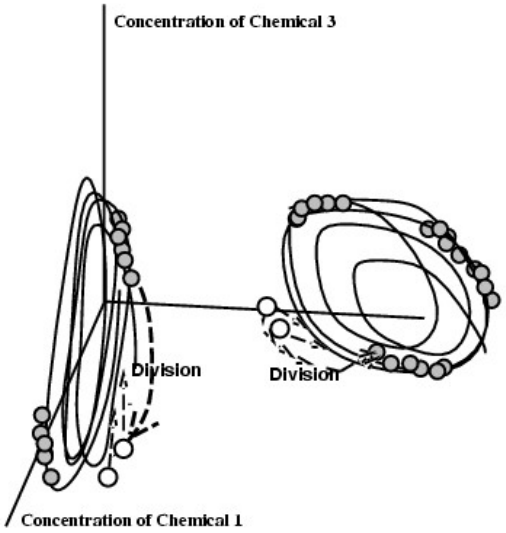


Assuming oscillatory dynamics as a single cell



formation of discrete types
with different chemical
compositions:
stabilize each other

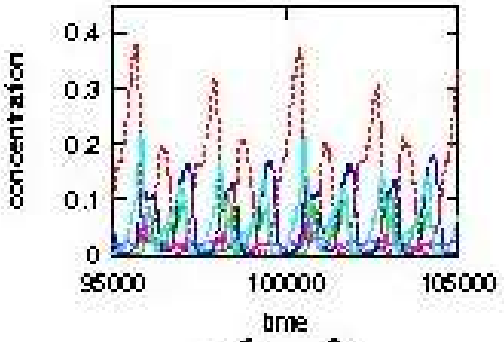
recursive production

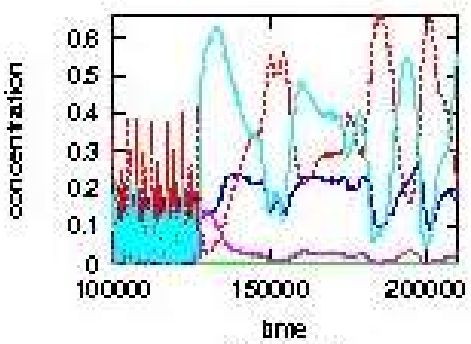


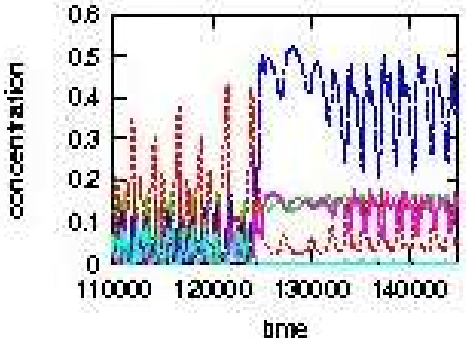
Kaneko, (c) Yomo 1997

Hierarchical differentiation from ‘stem cell’;by taking initially dynamics with instability (e.g., chaotic)
(higher order catalysis)

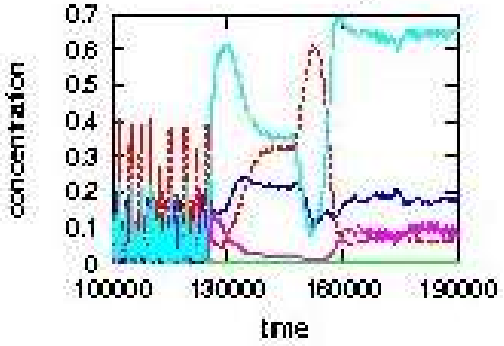
(III) if initial states show chaotic oscillation

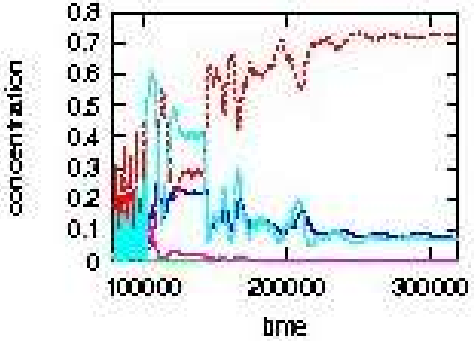






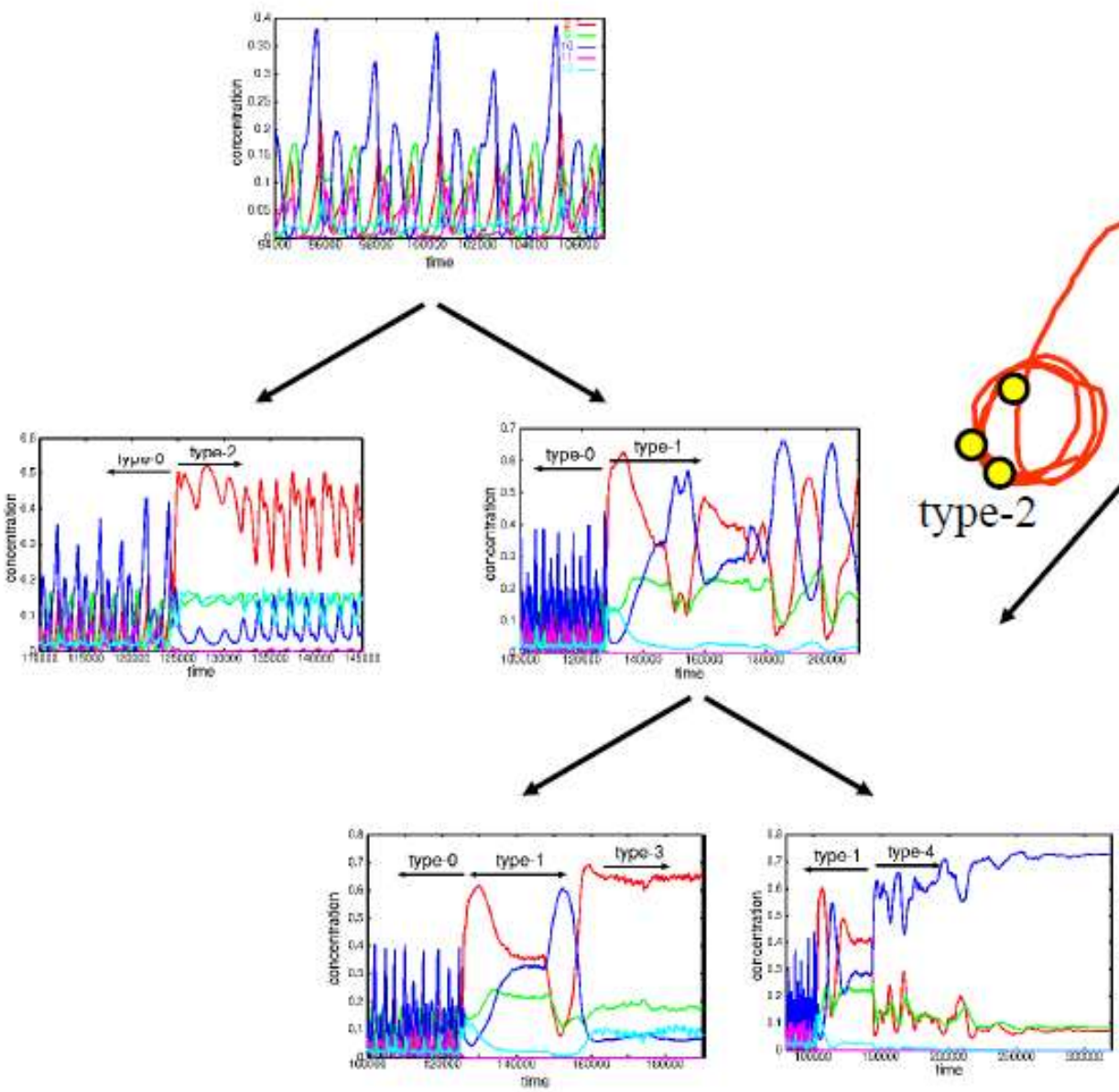
Chaos → slight change is amplified (butterfly effect)
It can produce stochastic behavior

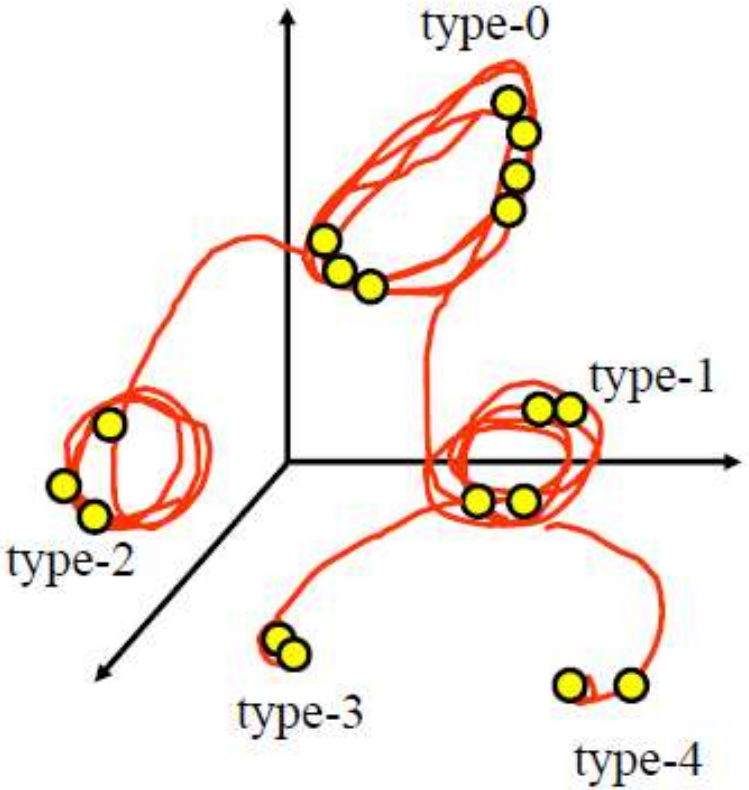




Furusawa & KK 1998,2001,2009

Hierarchical differentiation

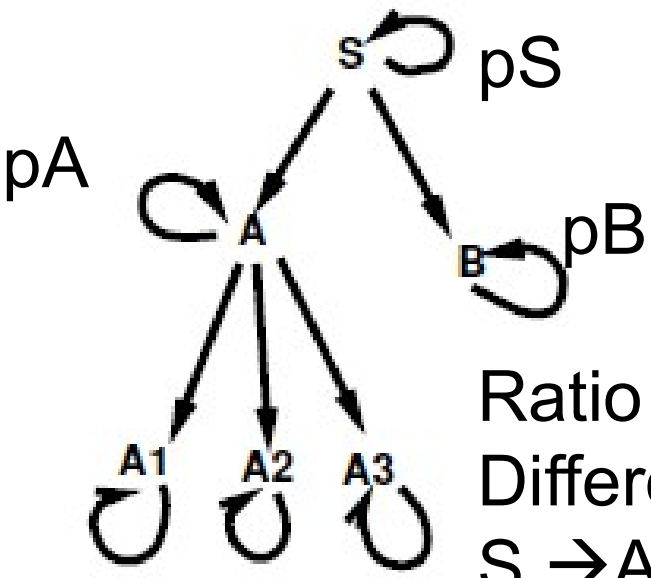


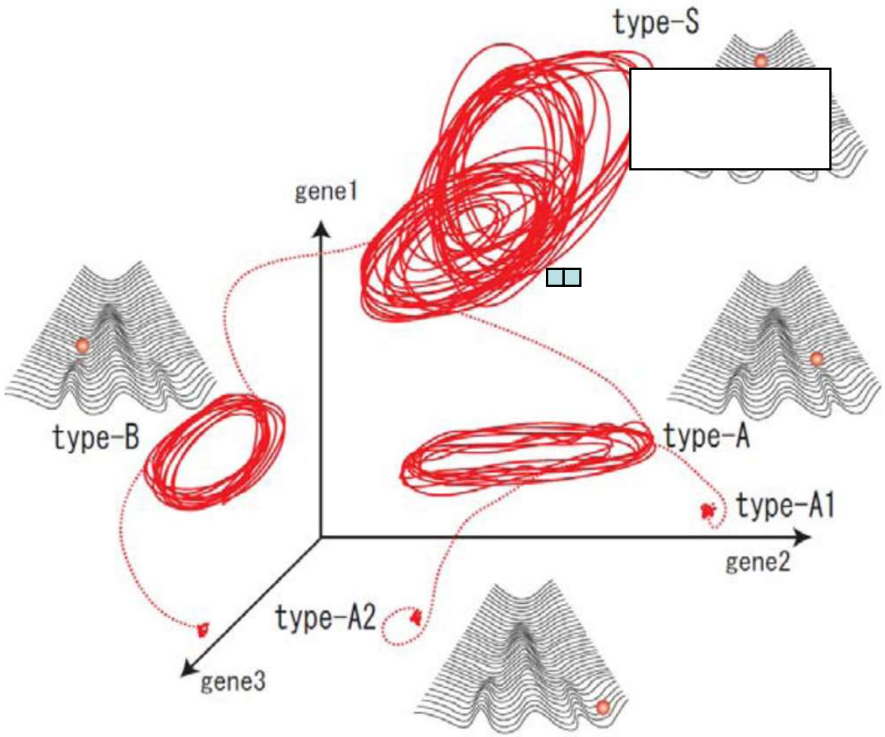


Initially chaotic dynamics
(chaos;
irregular oscillation
produce stochasticity
spontaneously
Still stable (attractor)

Furusawa & KK 98

Stable HierarchicalDifferentiation

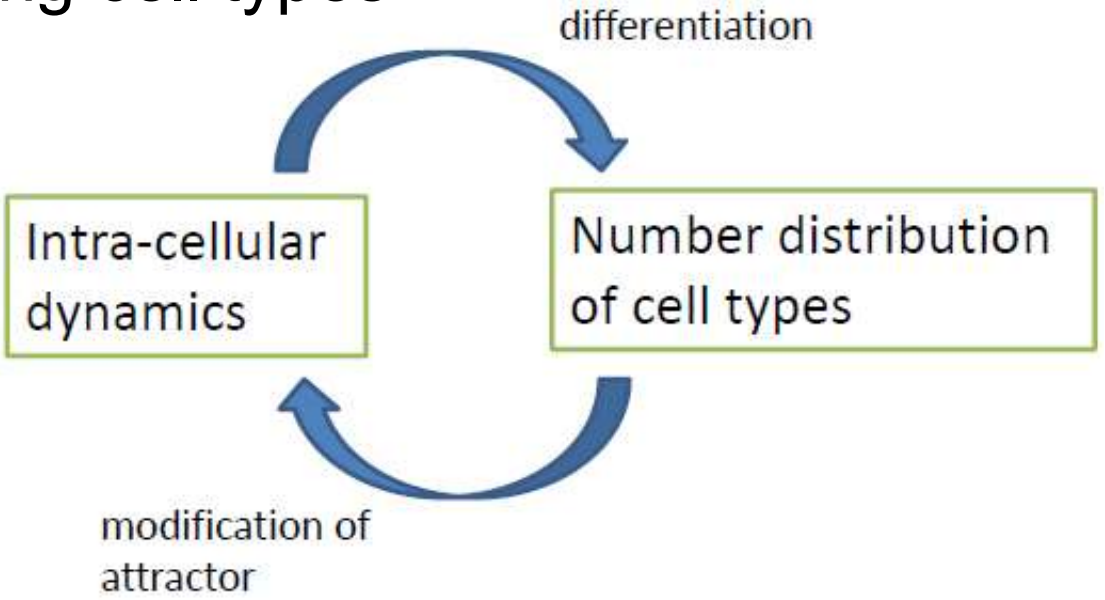




Ratio A decreased then
Differentiation ration
S →A is increased
Stable ratio among cell types

Hypothesis (Furusawa, KK 2001)

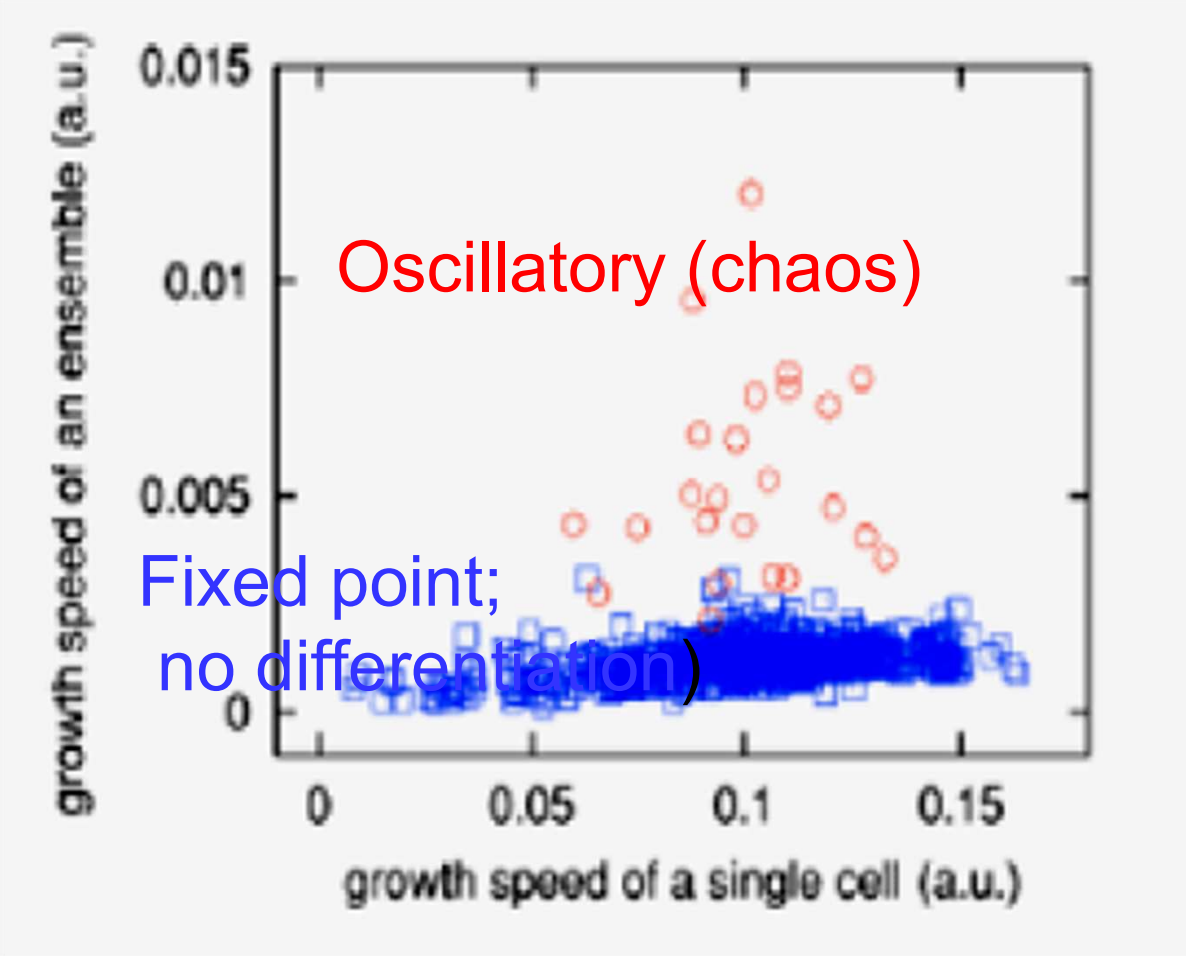
Gene Expression dynamics in stem cell = Oscillatory with itinerancy of several states
→cell-cell differentiation→
→robust differentiation



Loss of Pluripotency== Loss of such dynamics

Such chaotic dynamics are rather rare.
From a huge number of random networks; only some fraction of them show chaotic dynamics & differentiation

However, when differentiated, growth of an ensemble of cells is not decreased
such networks will be selected through evolution
Consistency between cell reproduction and multicellular growth



Furusawa,KK 2001PRL

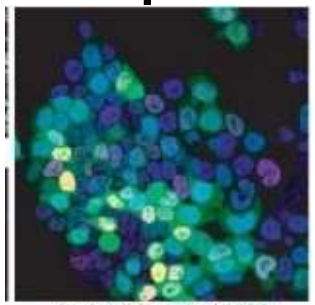
- Loss of pluripotency is characterized by

(i) Decrease in the diversity of expressed genes

initially diverse expressions – later specified in differentiated cells

(ii) Decrease in cell-cell variation

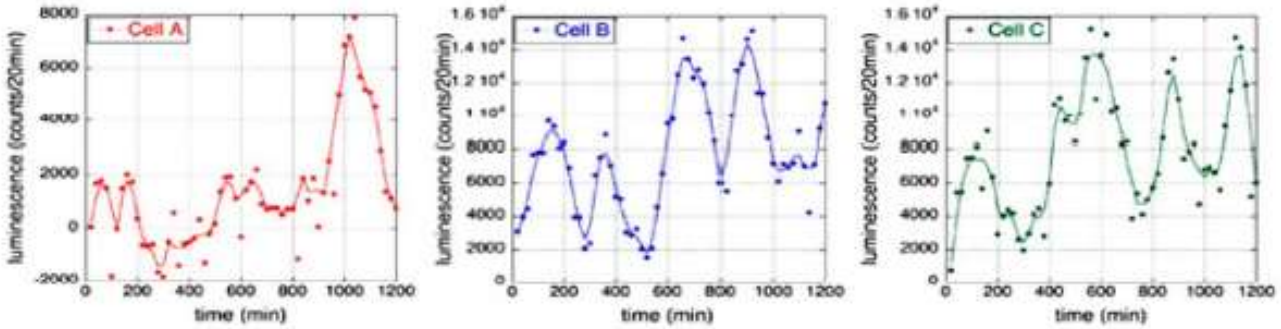
(exp. Heterogeneity confirmed)



(Chambers et al Nature07)

(iii) Loss of temporal variation in each cell

Oscillation in gene expression in stem cell:



Oscillation of Hes1 expression~4hr for ES
Lost when differentiated

Kobayashi et al. Genes Development 2009

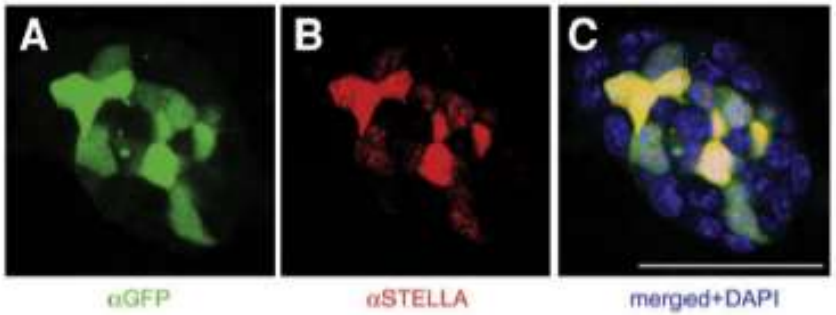
Gene expression dynamics Itinerancy over several states

Chang et al (Nature 08)

To recover Pluripotency → increase in degrees of freedom

(Furusawa,kk 01) ←?→ Yamanaka's iPS by expressing 4 genes

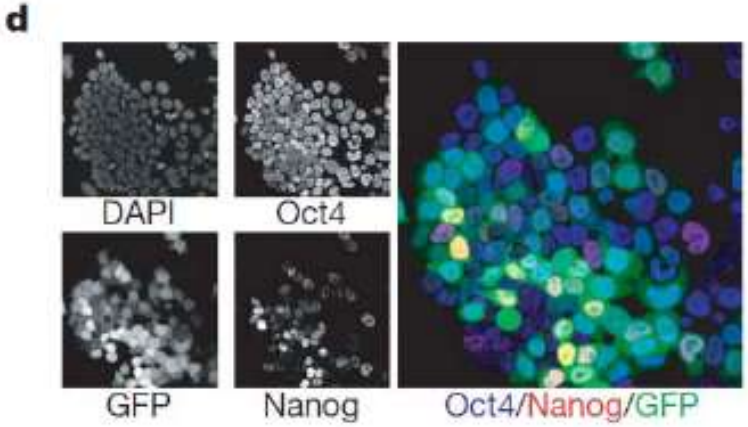
Heterogeneity in stem cell population



Stella expression

- ✓ a marker of pluripotency and germ cells

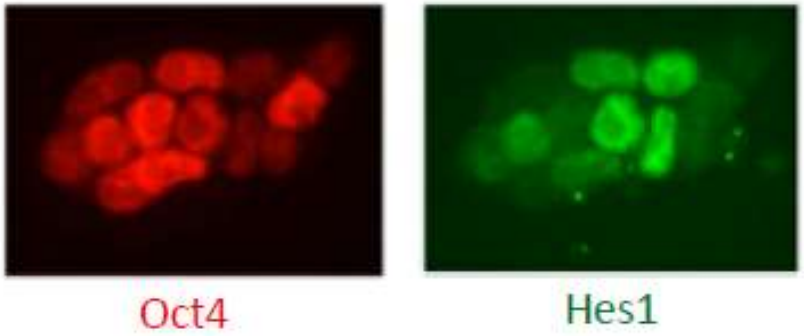
Hayashi et. al, Cell Stem Cell (2008)



Nanog expression

- ✓ considered as a core element of the pluripotent transcriptional network

Chambers et. al, Nature(2007)

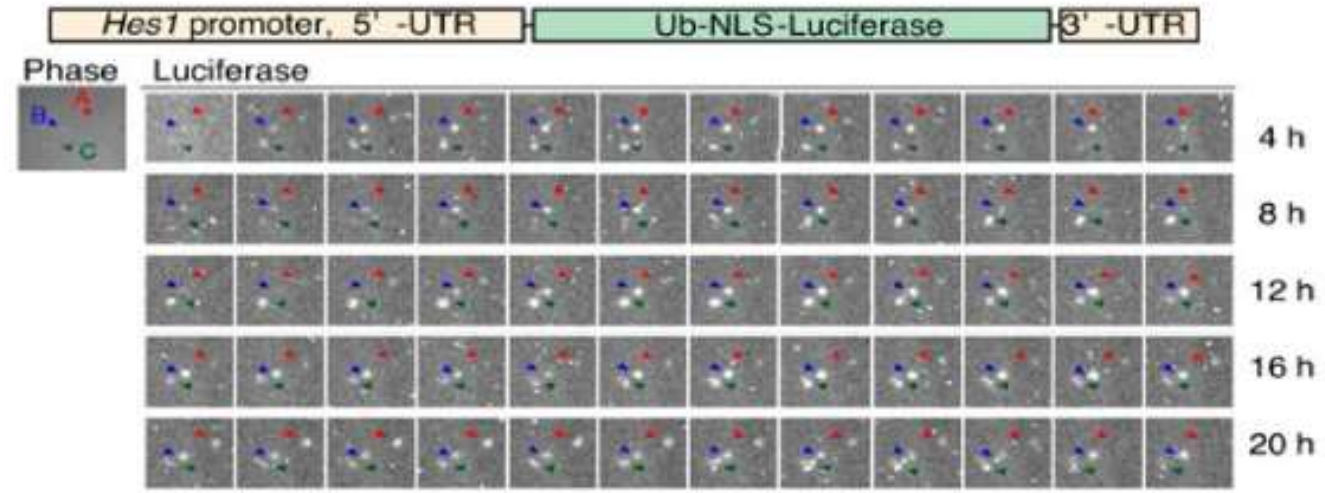


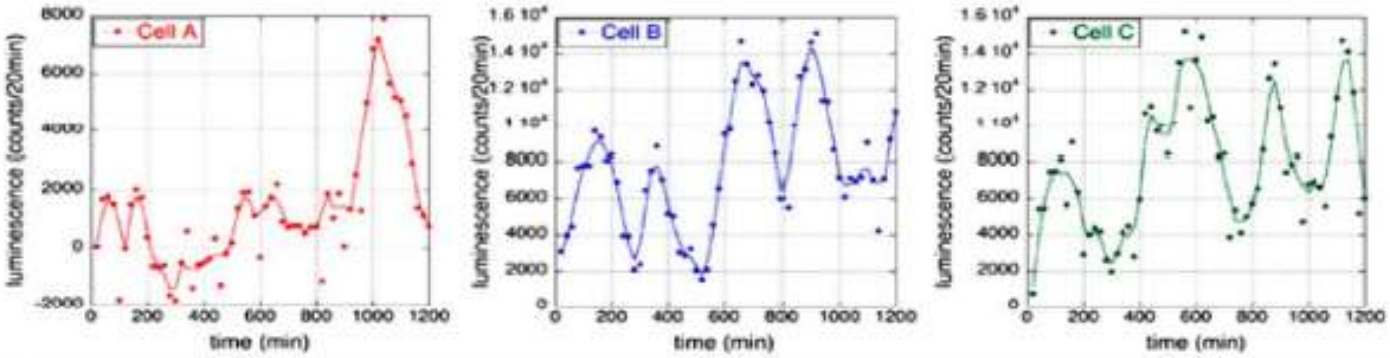
Hes1 expression

- ✓ a member of the bHLH transcriptional repressors that regulate cell proliferation and differentiation in embryogenesis

Kobayashi et. al, Genes & Development (2009)

Hes1 oscillation in ES cells





Expression of Hes1 exhibit an oscillatory behavior with a period of 3-5 hours.

This oscillation is Lost in differentiated cells!

Kobayashi et al. Genes Development 2009

To recover Stemness → increase in degrees of freedom (Furusawa,KK 2001) ←?→ Yamanaka's iPS (2006)by expressing 4 genes

J. theor. Biol. (2001) **209**, 395–416

Theory of Robustness of Irreversible Differentiation in a Stem Cell System: Chaos Hypothesis

CHIKARA FURUSAWA* AND KUNIHIKO KANEKO

8. Predictions

We believe that our results are universal in a class of dynamical systems satisfying minimal requirements of the developmental process. Although some of these universal features are not yet examined experimentally, we make some predictions here as general features commonly satisfied in real stem-cell systems. To conclude our paper, we summarize the predictions we can make using our model, and discuss the possibility of experimental verification.

8.4. IRREVERSIBLE LOSS OF MULTIPOTENCY CHARACTERIZED BY DECREASE OF COMPLEXITY IN CELLULAR DYNAMICS

While during the normal course of development, this loss of multipotency is irreversible, it is possible to recover the multipotency of a differentiated cell through perturbation, by changing the diversity of chemicals or the complexity of the dynamics. For example, by expressing a variety of genes compulsively in differentiated cells, the original multipotency may be regained. Note that, according to our model simulations, the basin of attraction of the stem cell is much larger than that of differentiated cells. This implies that by adding a large perturbation that results in the presence of a variety of chemicals in a cell, the cell de-differentiates back into a stem cell.

- **Revisit by Gene regulation Network Model**

1 diversification of cell types

2 Loss of Pluripotency ('time's arrow'?)

3 Robustness in cell types andTheir distribution

- Stem Cell

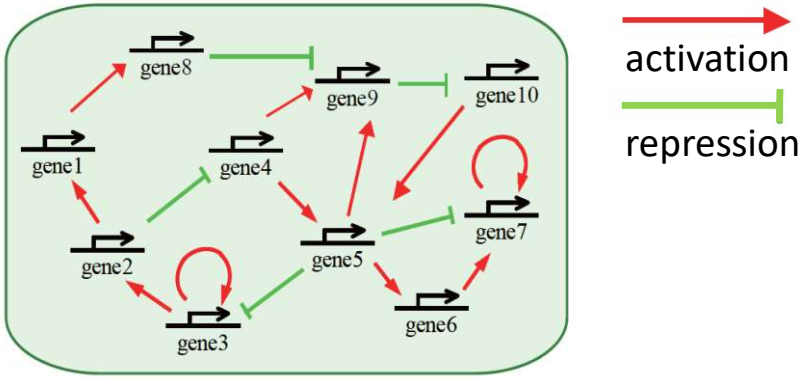
  Proliferation ---- Robust Differentiation --- Plastic

- How are these two opposing tendencies compatible?

  (Cf. Similar Question: Evolution, Brain, ….)

- Cell-cell interaction + Cell number increase

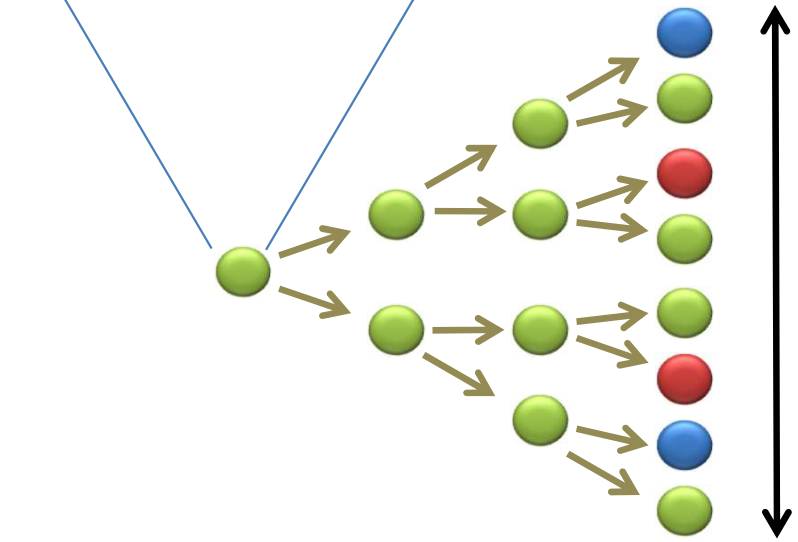
A simple model of multicellular development



activation

repression

Screening of regulatory networks that can generate cell-type diversity.



Extract general features independent of details of modeling.

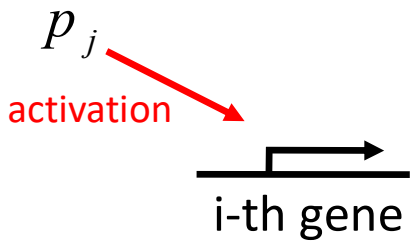
cell-cell interaction
(diffusion of proteins)

[1] N. Suzuki, C. Furusawa, and K. Kaneko, **PLoS One**, 6(11), e27232, 2011
[2] C. Furusawa and K. Kaneko, **Science**, 338(6104), 215-7, 2012

A cell model with on/off switching expression dynamics

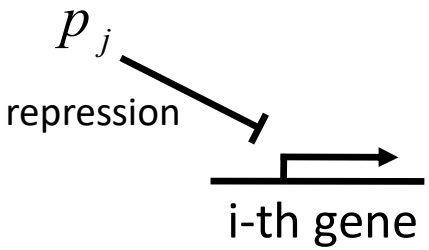
Cellular State
- mRNA expression levels $m_1, m_2, \cdots, m_n$
- protein expression levels $p_1, p_2, \cdots, p_n$

Dynamics of mRNA expressions

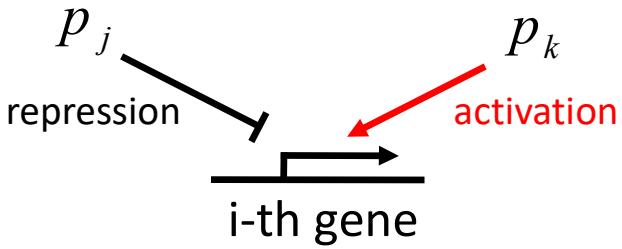


$$\frac{dm_i}{dt} = \frac{(p_j)^H}{1+(p_j)^H} - m_i$$

H: Hill coef. (=2)



$$\frac{dm_i}{dt} = \frac{1}{1+(p_j)^H} - m_i$$



$$\frac{dm_i}{dt} = \underbrace{\frac{1}{1+(p_j)^H}\frac{(p_k)^H}{1+(p_k)^H}}_{\text{synthesis}} - \underbrace{m_i}_{\text{degradation}}$$

Or we adopted thershold function model tanh(∑Jij pj)

A cell model with on/off switching expression dynamics

Dynamics of protein expressions

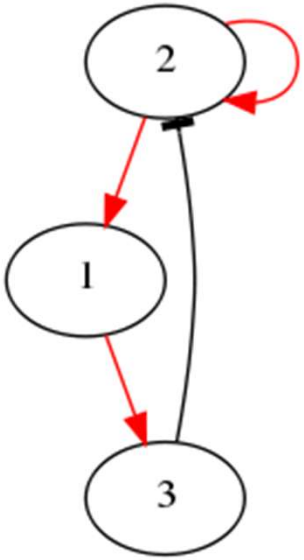
$$\frac{dp_i}{dt} = \alpha \cdot m_i - p_i + D_i\left(\overline{p}_i - p_i\right)$$

synthesis — degradation — diffution through the membrane

α, D_i : constant

$\overline{p}_i$: protein concentration in environment

For example:

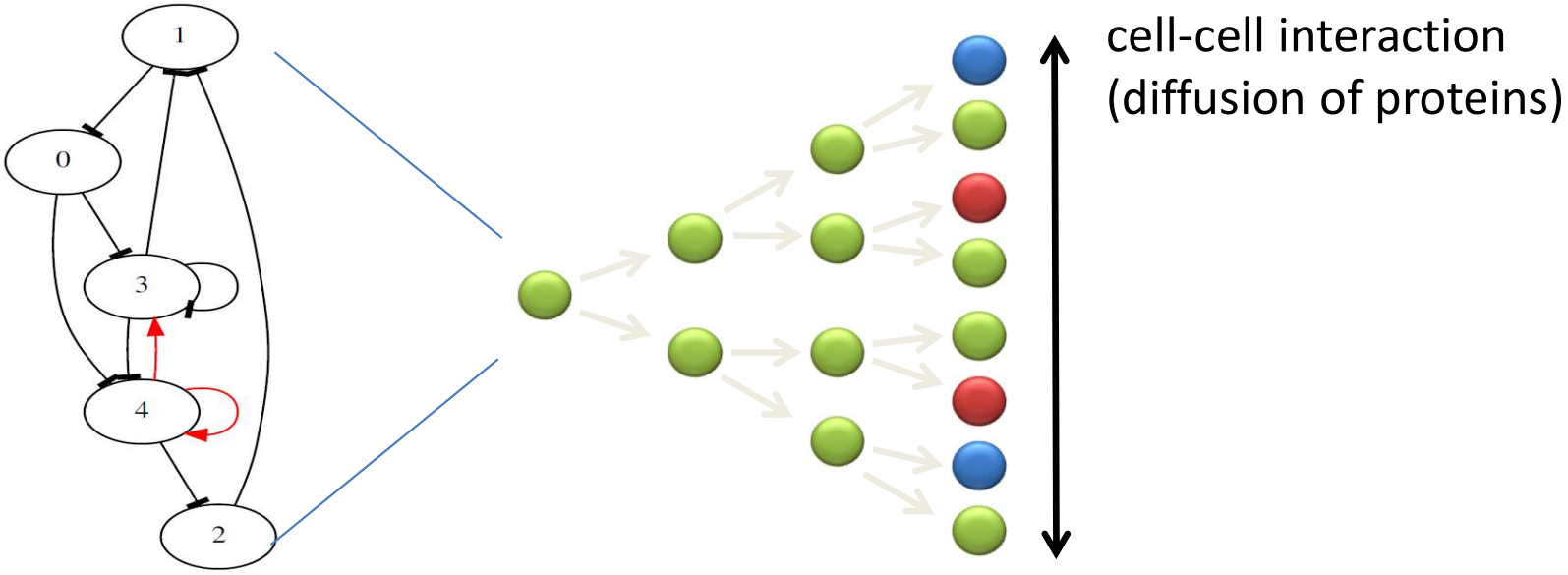


$$\begin{cases} \dfrac{dm_1}{dt} = \dfrac{(p_2)^H}{1+(p_2)^H} - m_1 \\ \dfrac{dm_2}{dt} = \dfrac{1}{1+(p_3)^H}\dfrac{(p_2)^H}{1+(p_2)^H} - m_2 \\ \dfrac{dm_3}{dt} = \dfrac{(p_1)^H}{1+(p_1)^H} - m_3 \\ \dfrac{dp_1}{dt} = \alpha m_1 - p_1 + D_1\left(\overline{p}_1 - p_1\right) \\ \vdots \\ \vdots \end{cases}$$

Screening regulatory networks

Screening regulatory networks that can maintain multiple cell types by simulating all possible regulatory networks (∼1.4×10^8 networks)

✓ 5 genes, 10 regulatory paths



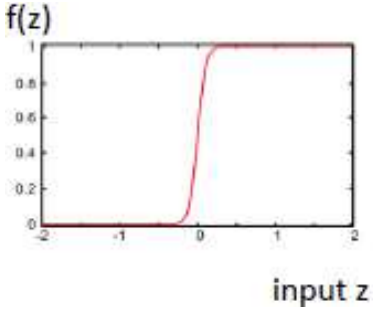
[1] N. Suzuki, C. Furusawa, and K. Kaneko, **PLoS One**, 6(11), e27232, 2011
[2] C. Furusawa and K. Kaneko, **Science**, 338(6104), 215-7, 2012

Further confirmation of our chaotic itinerancy hypothesis

☆Dynamics of i-th mRNA abundance in k-th cell:

$$\frac{dm_i^k}{dt} = f\left(\sum_j W_{ij} p_j^k\right) - m_i^k \qquad \text{with} \quad f(z) = \frac{1}{1+e^{-\mu \cdot z}}$$

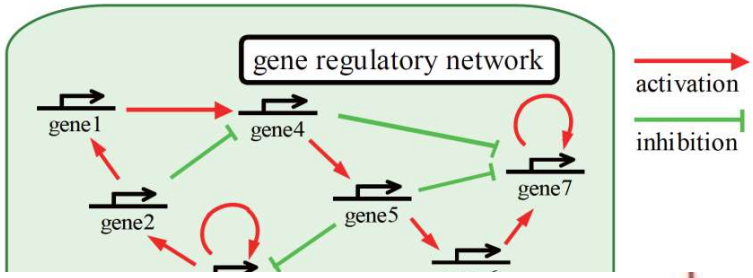
synthesis of mRNA　　degradation

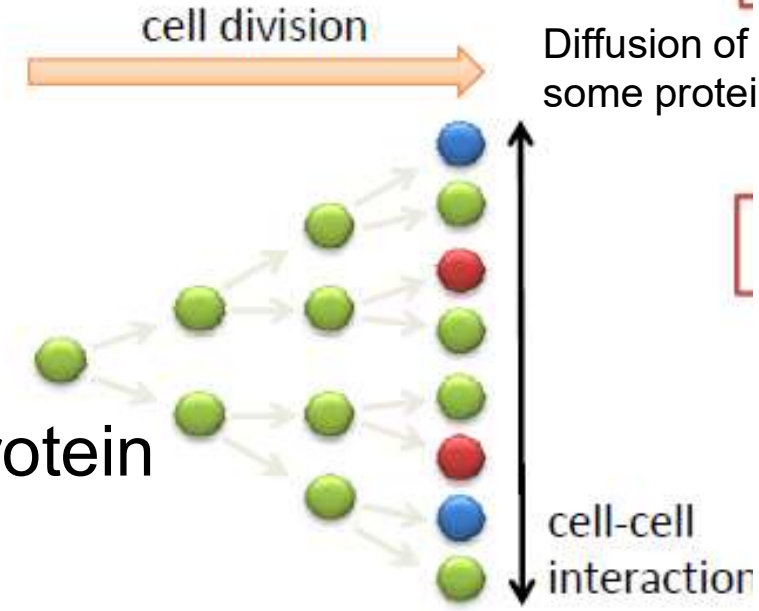


☆Dynamics of i-th protein abundance in k-th cell:

$$\frac{dp_i^k}{dt} = \alpha \cdot m_i^k - p_i^k + D_i\left(\overline{p}_i - p_i^k\right)$$

synthesis　degradation　transport through the membrane



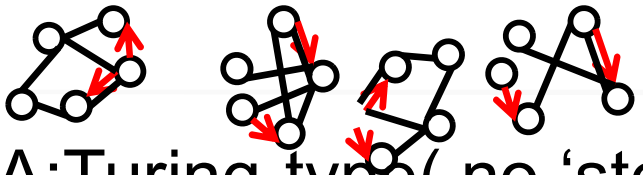


Increase in the cell number by division
In division put some noise in m,p between cells

Cell-cell interactions: diffusion of some protein

C.Furusawa、N.Suzuki

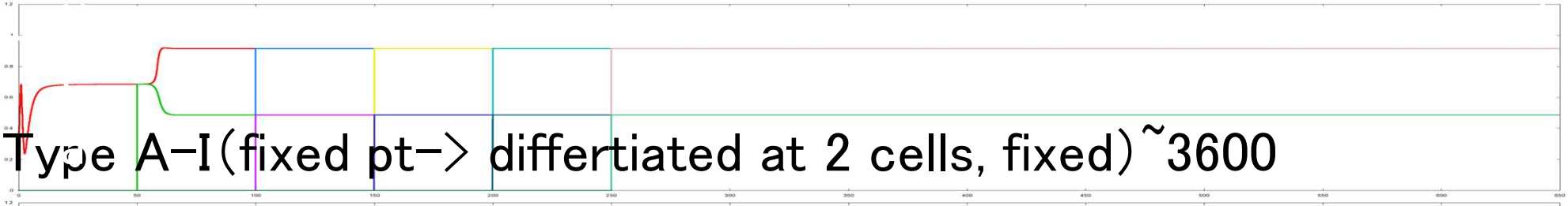
Simulations of all GRN with 5 gene and 10 paths



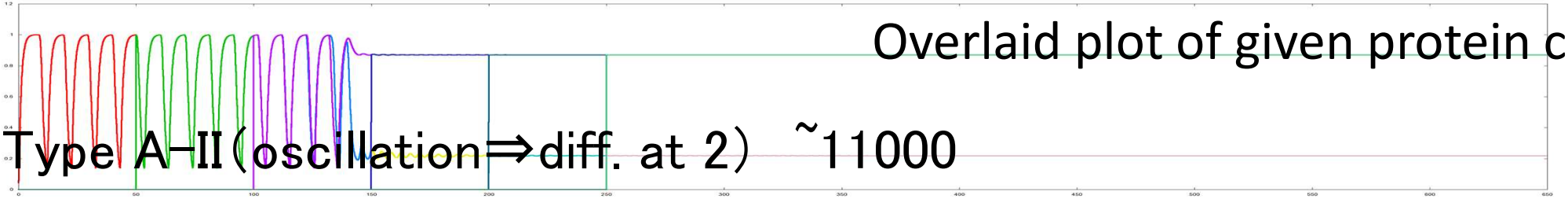
145269760 networks

Differntiated at 32 cells → (14,997)

A:Turing-type(no ‘stem cells’ both with proliferation & differentiation



Type A-I(fixed pt-> differtiated at 2 cells, fixed)~3600

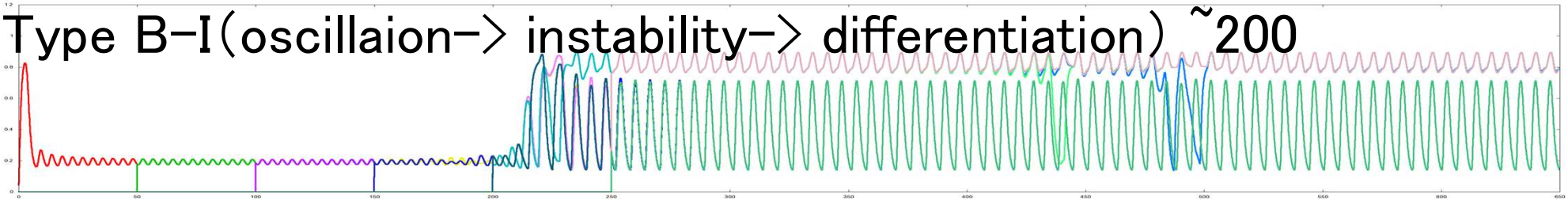


Overlaid plot of given protein conc

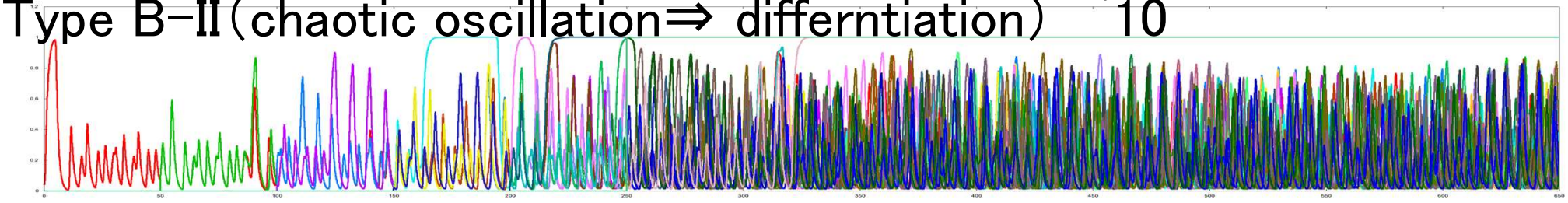
Type A-II(oscillation⇒diff. at 2) ~11000

B:Itinerant dynamics+ Interaction＝＞stem cell

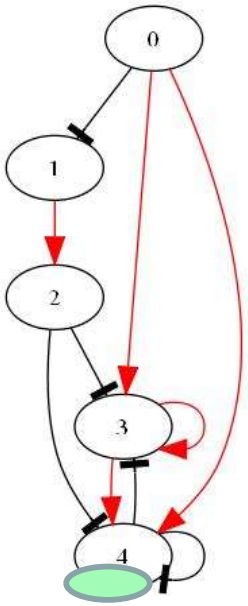
Type B-I(oscillaion-> instability-> differentiation) ~200



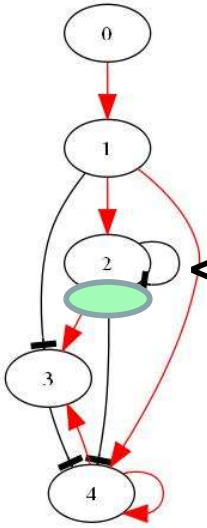
Type B-II(chaotic oscillation⇒ differntiation) ~10



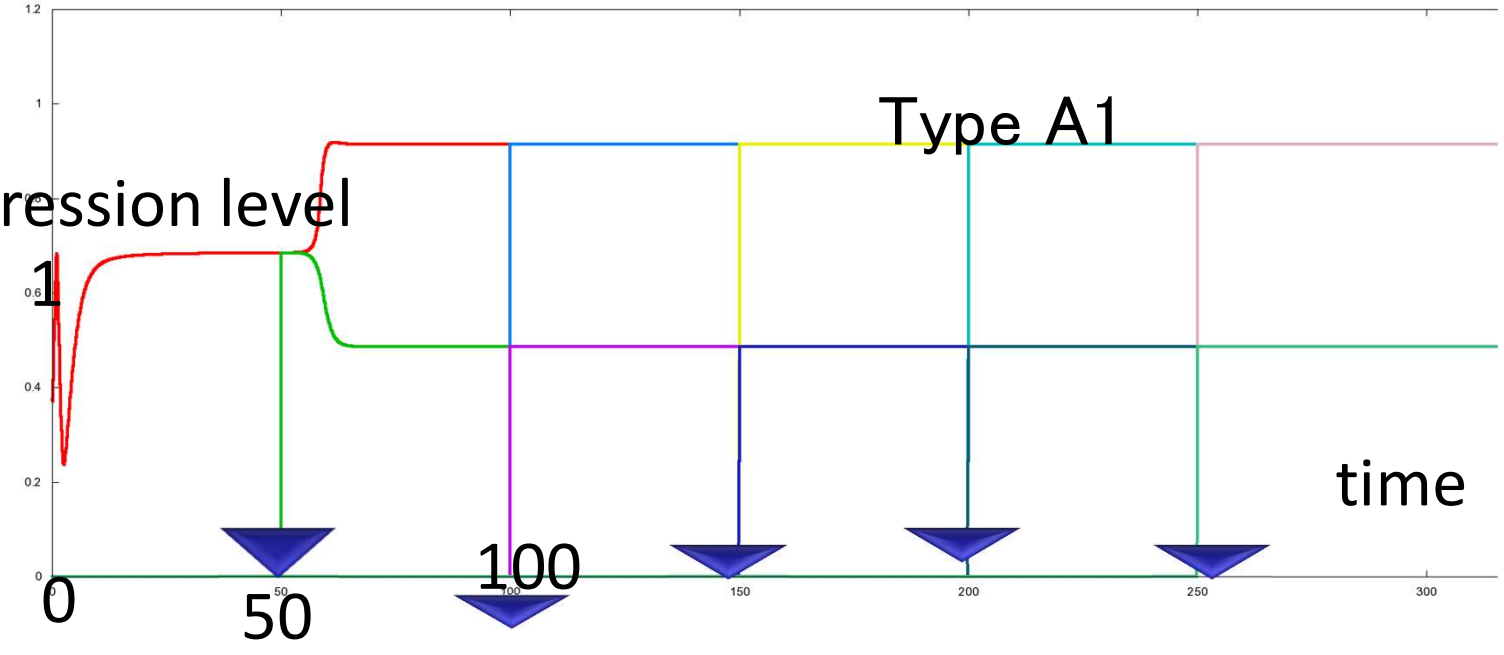
TypeA: Turing (diffusion of inhibitor + activator)

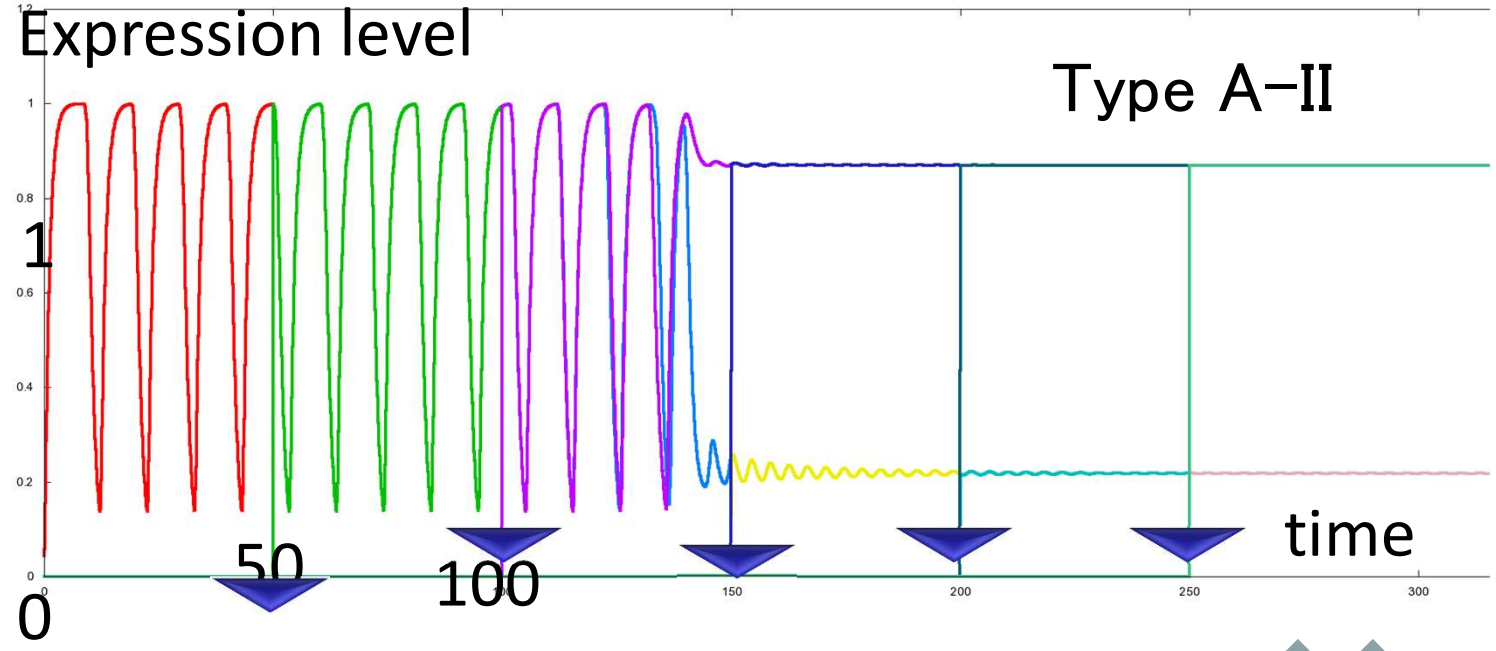


^diffusive



<-diffusive

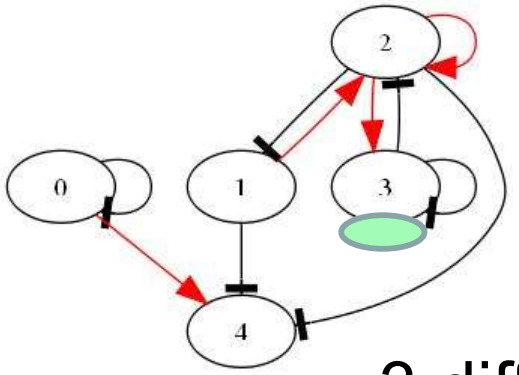




No cells that satisfy both prolifereation and differentiation stem

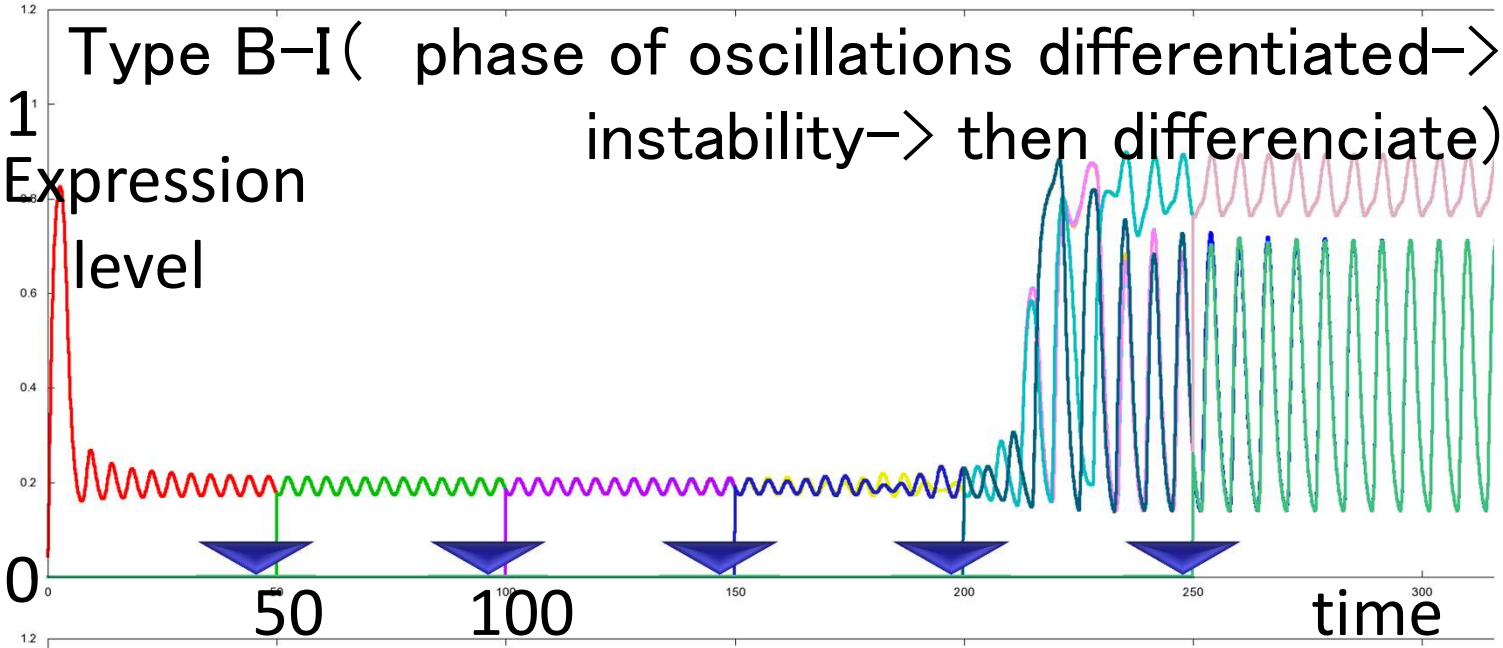
3 typeB＝Stem-cell=Proliferation+differentiation

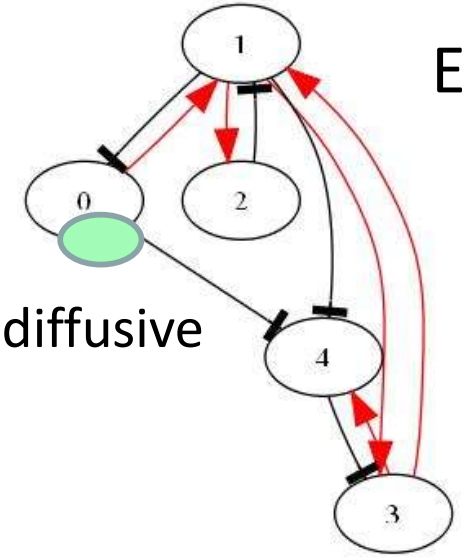
more than single differentiations observed with the increase of cell number



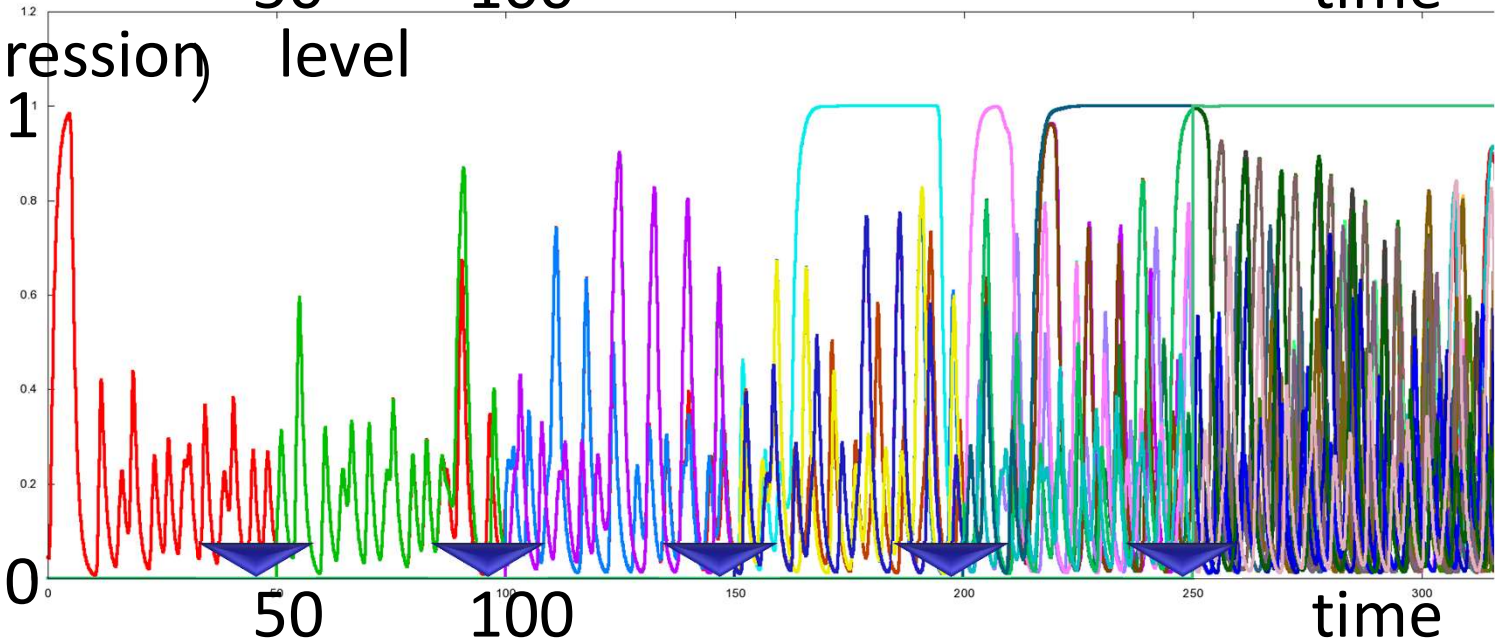
3:diffu sive

Type B-I(phase of oscillations differentiated-> instability-> then differenciate)



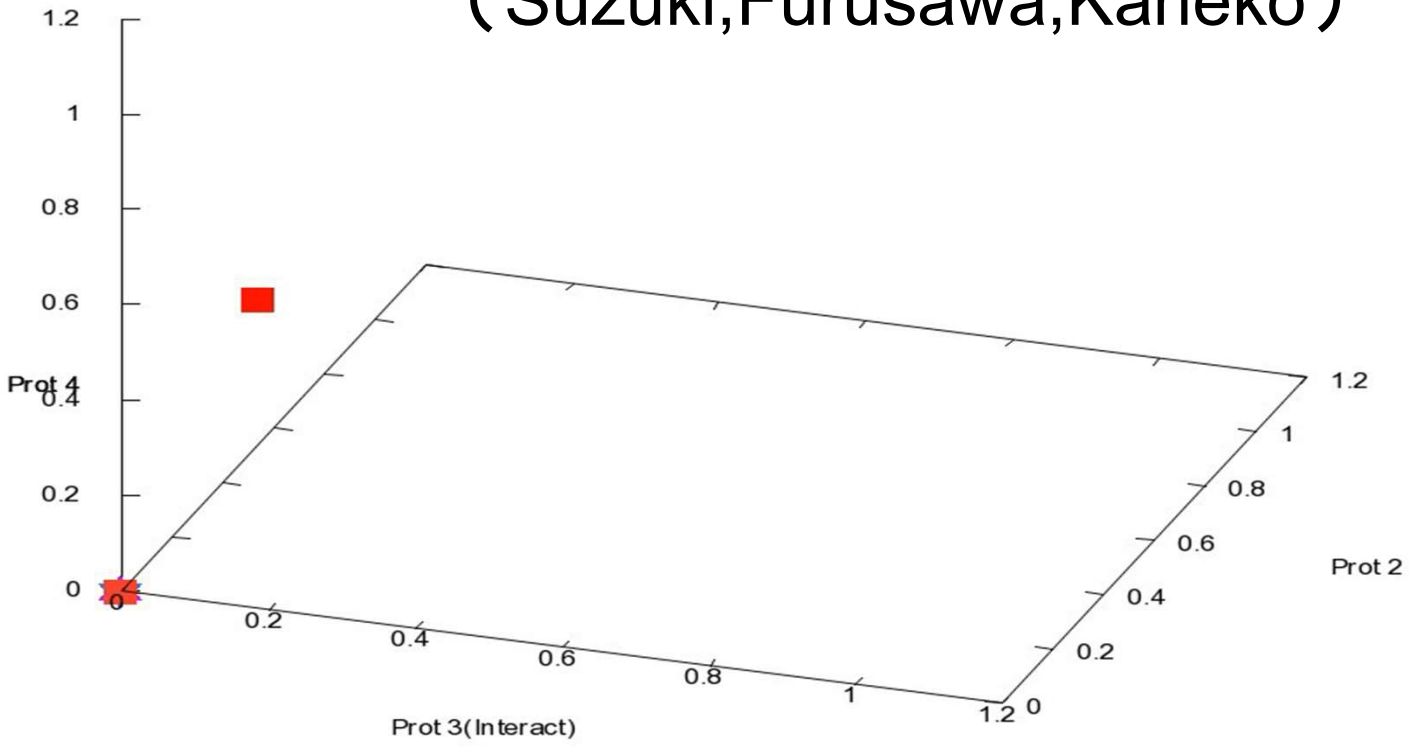


0:diffusive

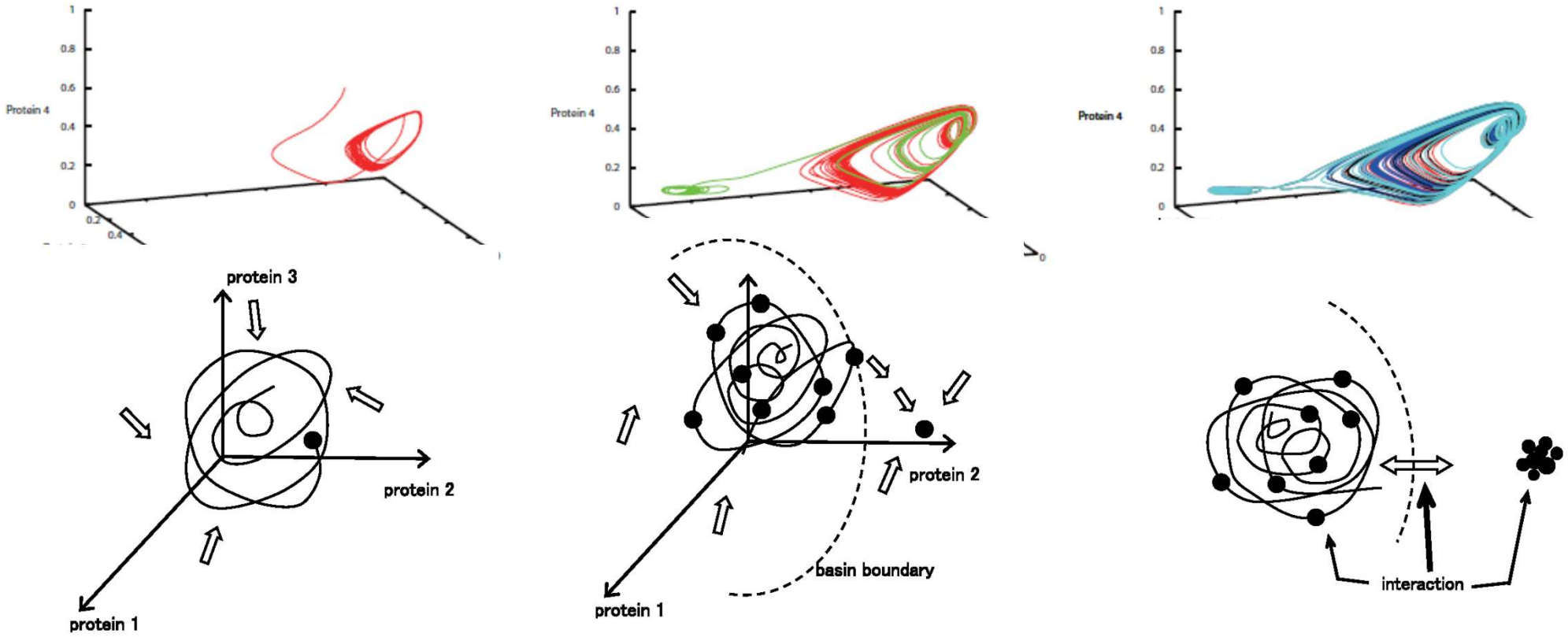


Time0

Videos available at youtube
（Suzuki,Furusawa,Kaneko）



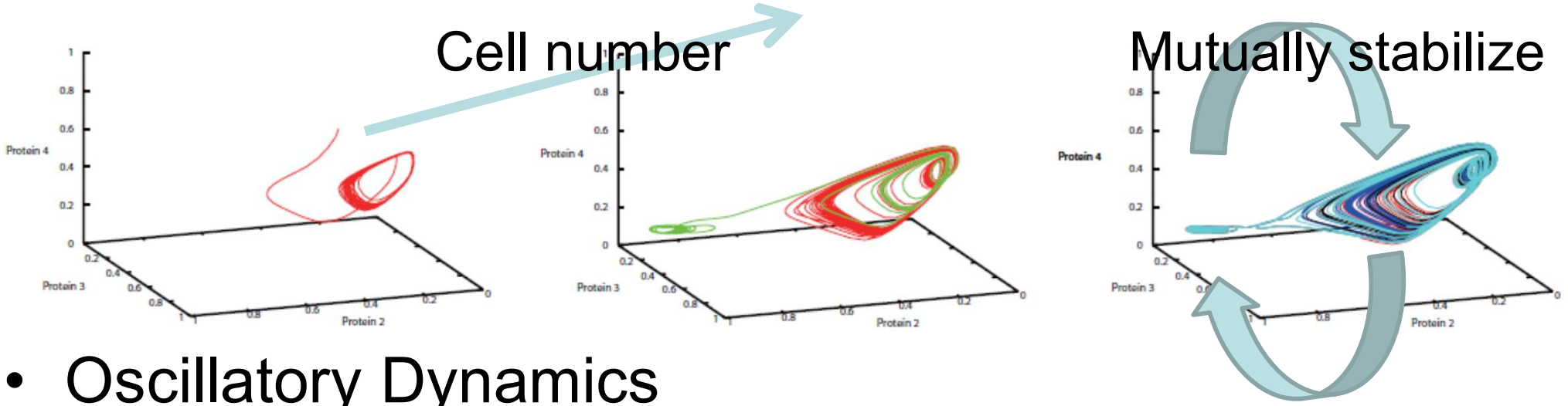
Differentiation by bifurcation with effective parameter change in coupled dynamical systems



Cell-cell interaction → changes effective bifurcation parameter → lead to distinct cellular states → distribution of different states leads to consistent parameter changes

Cell number increase→ Selection of states

Dynamical Systems Mechanism



- Oscillatory Dynamics

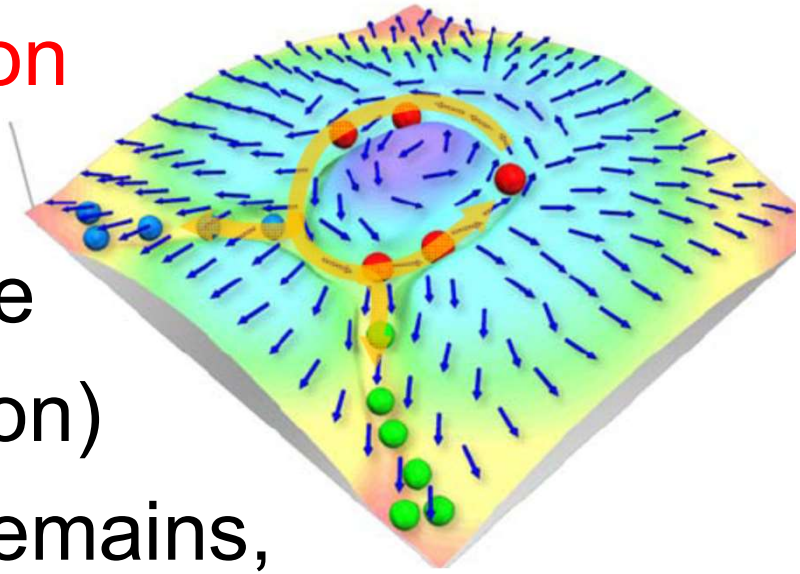
→Desynchronized irregular oscillation

by cell-cell interaction

→ some cells switch to a novel state

(bifurcation & stabilized by interaction)

When desynchronized oscillation remains, differentiation continues.

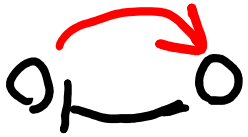


Rate of differentiation or self-renewal depends on the number ratio of each cell type → its regulation

- Common **Network Structure** for 'Stemness'

--Combination of 'Oscillation & 'Switch Modules'

☐Oscillation: (Plural) negative feedback loops

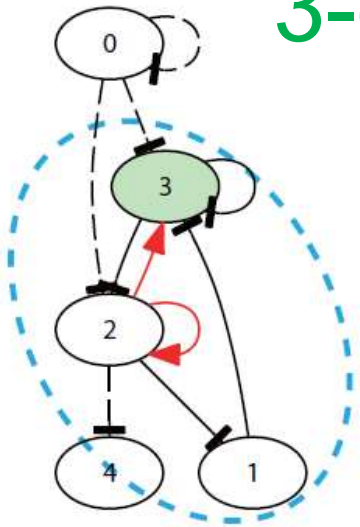


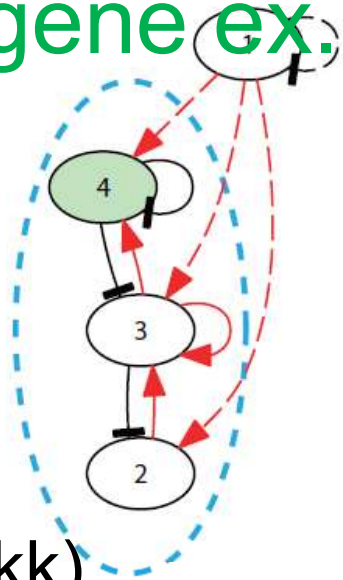
Plural is better for complex oscillation, needed to keep desynchronization

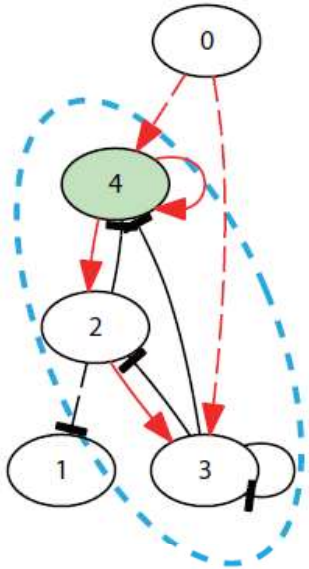
☐Switch： Positive

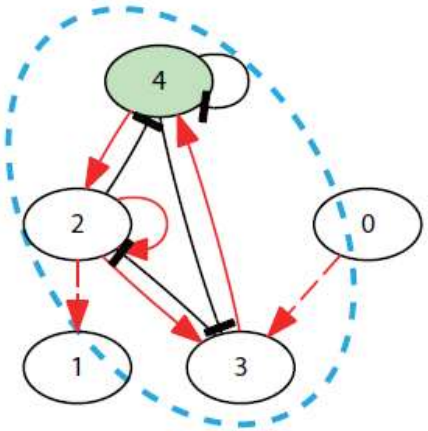
（Turing type）

3-gene ex.

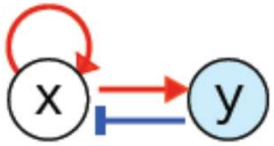


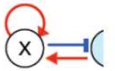


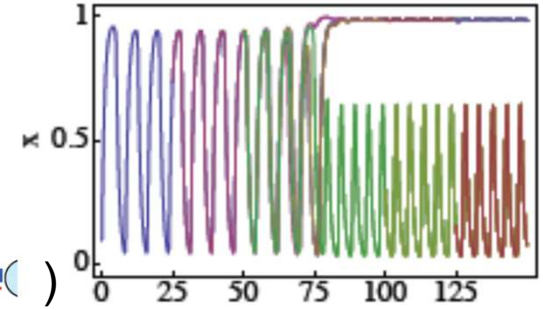


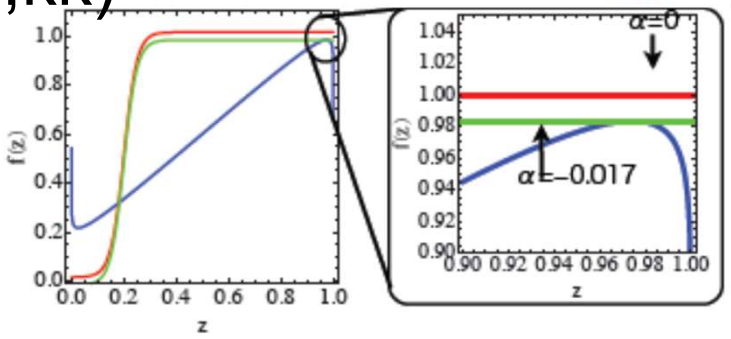


Simplest 2-gene model (Goto,kk)



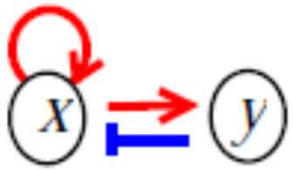
(Similar for )





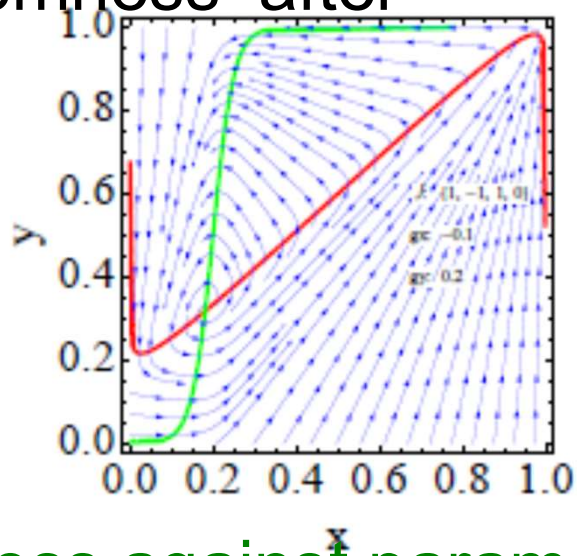
Interaction → Oscillatory to Fixed point

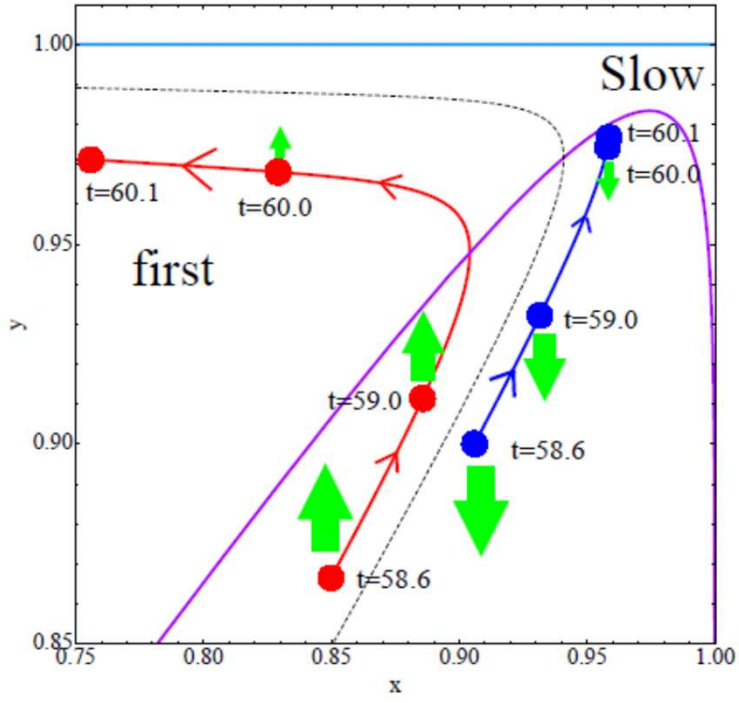
Simplest=2, but in a rather small parameter regime (cannot keep stemness after few times)



$g_x = -0.1$

$g_y = 0.2$



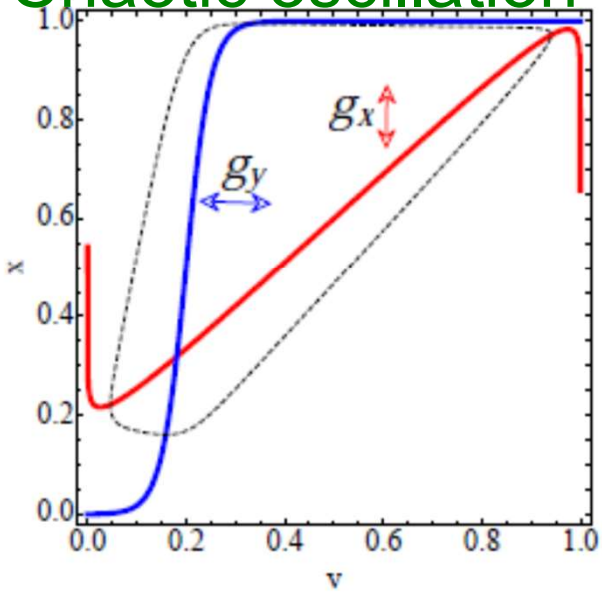


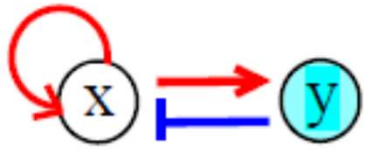
dx/dt=0 ····· single cell

dy/dt=0 two cells

effect of diffusion term

To have robustness against parameter change, and to preserve stemness after many divisions Chaotic oscillation is preferable

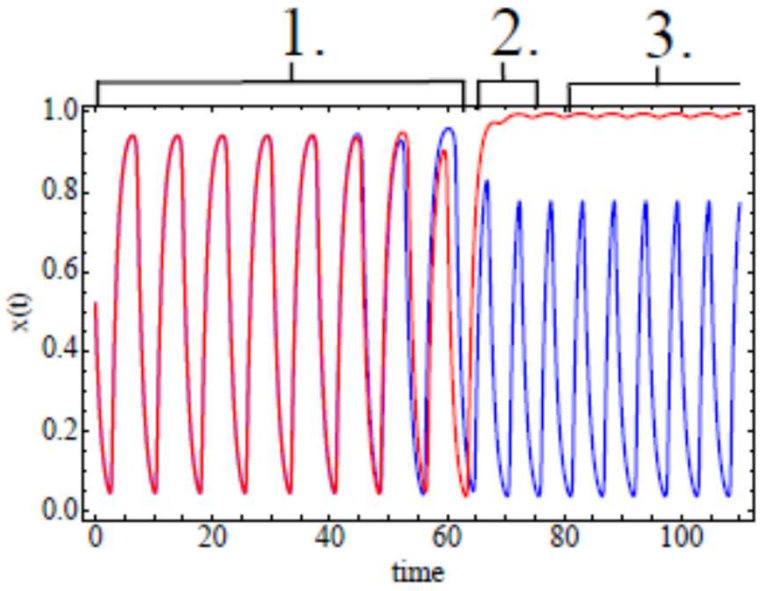




dx/dt=0

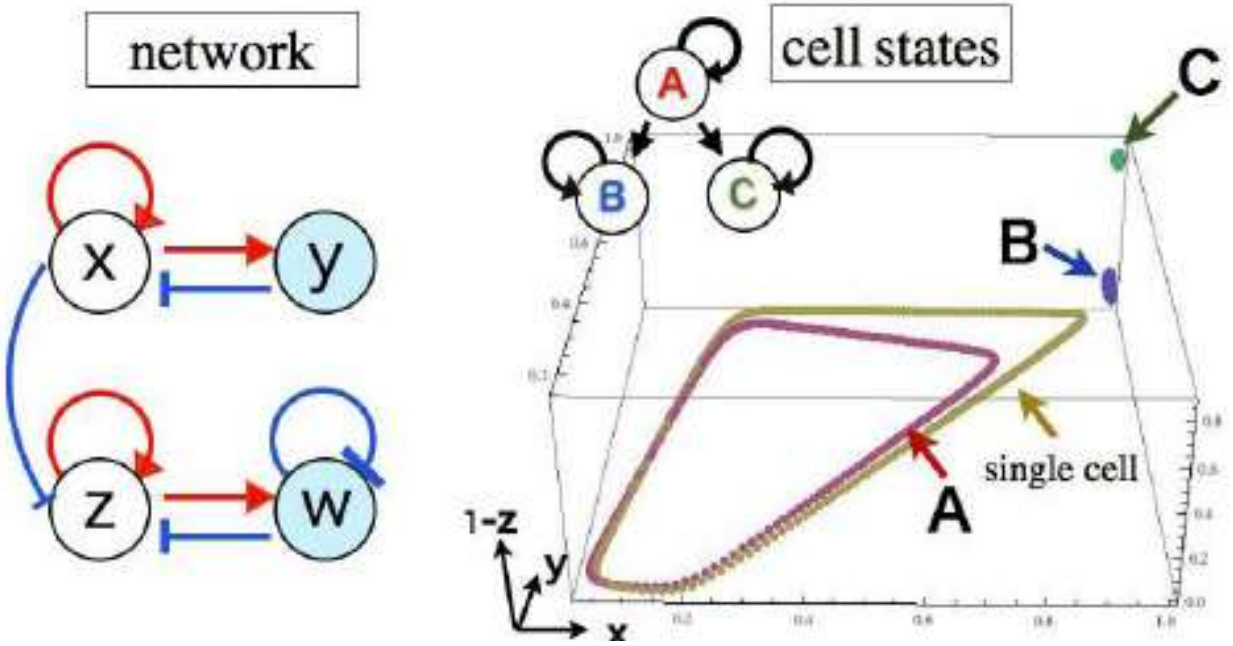
dy/dt=0

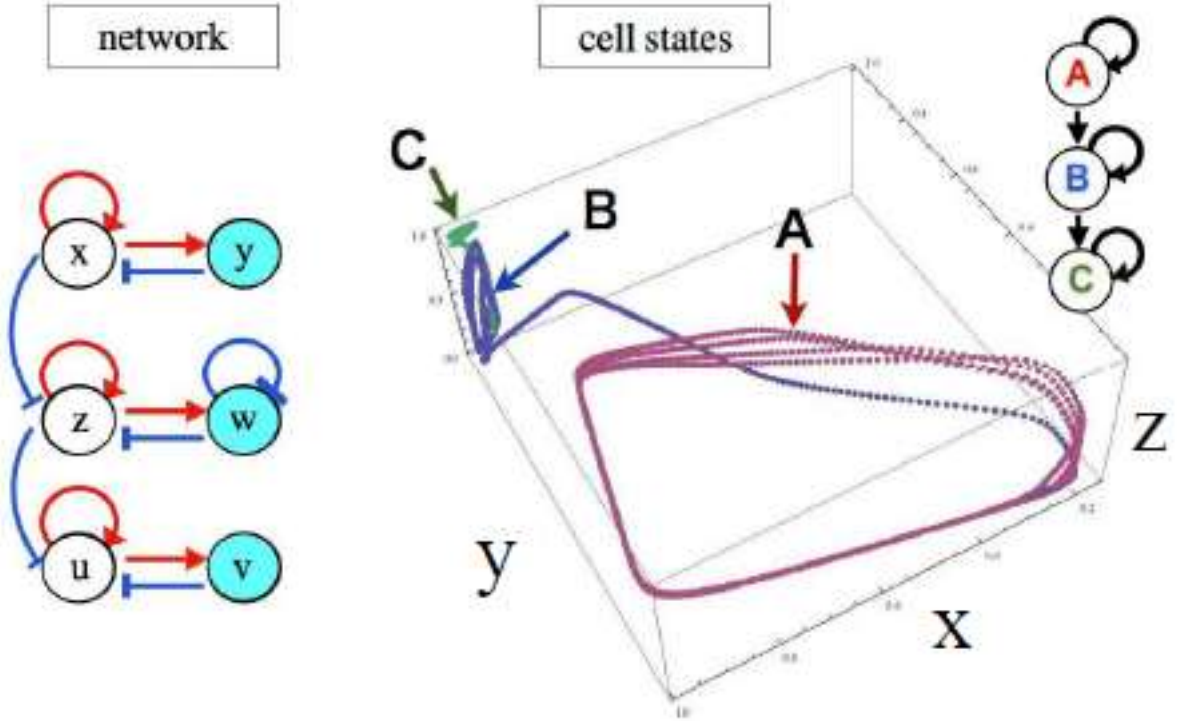
stady state (N=1)



1.Desynchronization

One can generate complex, hierarchical differentiation





Summary:

Itinerant (chaotic) Dynamics → Pluripotency

Cell-cell interaction → Differentiation to lose pluripotency

Alternative view;

multistable 1-cell dynamics + switch by noise

(indeed commonly found if higher noise) but

(i)robustness in number-ratio of cell-types: difficult

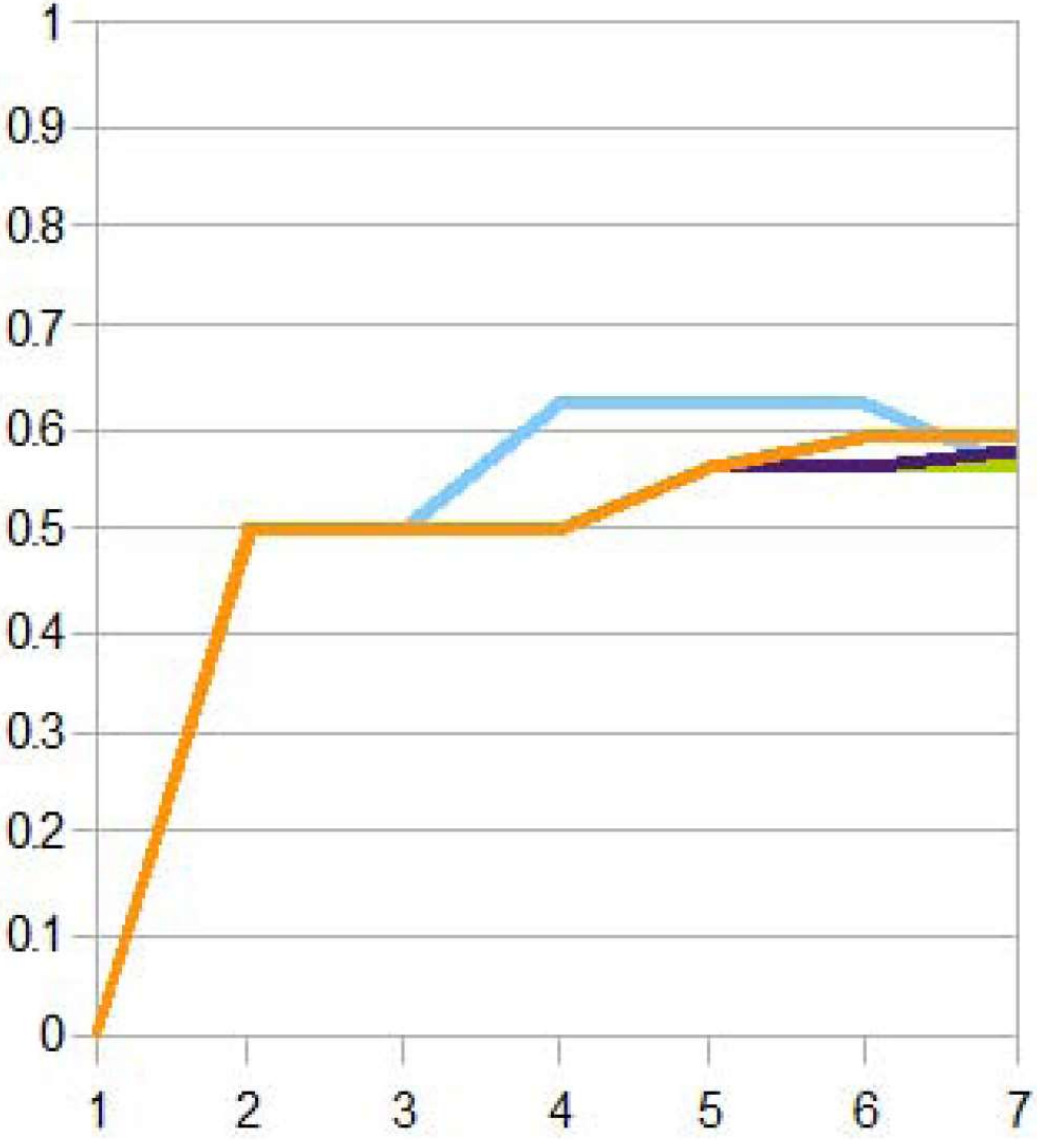
(ii) needs fine tuning of noise amplitude (especially for differentiation to several types)

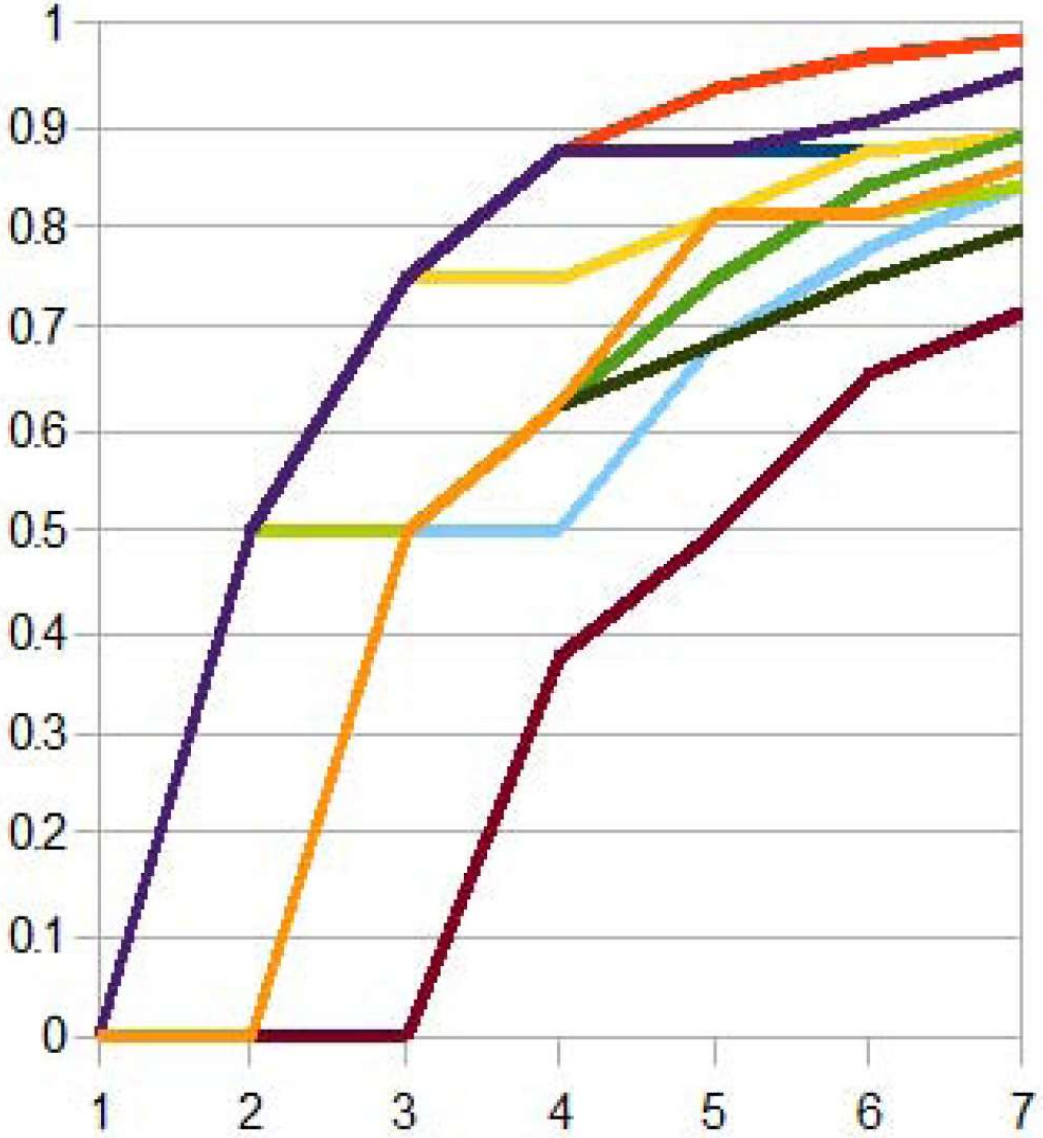
Ours: (i) spontaneous cell number ratio regulation

(ii)Diversification with hierarchic differentiation easy

(iii)Evolvability: Chaotic Dynamics have higher potentiality for evolution to diversification

(iv) Experimental support (?)


1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7


1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7

Theory of coupled dynamical systems (to be omitted)

$$\frac{dx_i^m}{dt} = f^m(\vec{x_i}) + D^m(X^m - x_i^m)$$

$$\frac{dX^m}{dt} = -D^m \sum_j (X^m - x_j^m)/V.$$

7.6 Dynamical systems model of cell differentiation through cell-cell interac

the environment occur immediately, adiabatic elimination with dX^m/d gives $X^m = (\sum_j x_j^m)/N$, where N is the number of cells. Therefore, w tain

$$\begin{aligned}\frac{dx_i^m}{dt} &= f^m(\vec{x_i}) + D^m(\frac{\sum_j x_j^m}{N} - x_i^m) \\ &= f^m(\vec{x_i}) + I_i^m,\end{aligned}$$

where

$$I_i^m = D^m(\frac{\sum_j x_j^m}{N} - x_i^m) = M^m - D^m x_i^m.$$

Here, I_i^m represents the effect of cell-cell interactions that the i-th ce ceives, and M^m represents the mean field "interaction" that all cells

I: Stability of uniform state

If all cell states are identical, satisfying $x_i^m = x_j^m$, then the interaction term I_i^m is 0. Therefore, the uniform state in which all cells take the identical state and change commonly by $dx^m/dt = f^m(\vec{x_i})$ is always the solution to this equation. Is this solution stable? For this purpose, let us see whether a small difference between the states of two cells δ^m will increase over time. Using the Jacobi matrix $J_{m\ell} = \partial f^m/\partial x_\ell$, the change in δ^m can be represented as follows:

$$\frac{d\delta^m}{dt} = \sum_\ell J_{m\ell}\delta^\ell - D^m\delta^m. \tag{7.12}$$

The stability of the uniform state can be evaluated by the eigenvalues of the matrix consisting of the Jacobian matrix minus D^m in the diagonal components. If the real parts of the eigenvalues are all negative, then its uniform state is stable. If D^m is constant for all components, this eigenvalue is simply a subtraction of D^m from the eigenvalue of the original Jacobian matrix, so the uniform state is always stable if the original fixed point is stable (all real

For simplicity, we first consider the case in which two types of cells arise, whose numbers are $N_1 = \rho_1 N$ and $N_2 = \rho_2 N$, respectively. Then, we obtain

$$\frac{dx_1^m}{dt} = f^m(\vec{x_1}) + D^m \rho_2 (x_2^m - x_1^m) \tag{7.13}$$

$$\frac{dx_2^m}{dt} = f^m(\vec{x_2}) + D^m \rho_1 (x_1^m - x_2^m). \tag{7.14}$$

In this model, if $x_1^m \neq x_2^m$, then the effect of the interaction works in the opposite direction in type 1 and type 2 cells. For example, consider the case in which each cell type falls to a fixed point, $\{x_1^{m*}\}$ and $\{x_2^{m*}\}$, and the cells with different states stabilize each other. Then, in addition to the intracellular dynamics of single cells, type 1 and type 2 cells are affected by interactions $I_1^m = D^m \rho_2 (x_2^{m*} - x_1^{m*})$ and $I_2^m = D^m \rho_1 (x_1^{m*} - x_2^{m*})$, respectively. If we consider the interaction term I as a bifurcation parameter, the fixed point is destabilized in the uniform state with $I = 0$, and a stable

Example 7.1: Turing instability: In a two-component system, consider the case where the fixation point is stable in a single cell, where $D^2 = D > 0$ is given for the second component and $D^1 = 0$ for the first component. If the Jacobi matrix at a fixed point is $J = \begin{pmatrix} a & b \\ c & d \end{pmatrix}$, the eigenvalue λ is the solution of $\lambda^2 - (a+d)\lambda + (ad - bc) = 0$. Since this real part must be negative because it is a stable fixed point, its conditions are $a + d < 0$ and $(ad - bc) > 0$. On the other hand, the eigenvalue of the matrix $J - D\delta^2$ is the solution of $\lambda^2 - (a+d-D)\lambda + (a(d-D) - bc) = 0$, because we only need to make d above $d - D$. For the interaction to destabilize the uniform state, the real part of this eigenvalue must be positive. Since now $a+d < 0$, $D > 0$, and then $a + d - D < 0$. Then, destabilization requires $a(d - D) - bc < 0$, that is, $aD > (ad - bc) > 0$. Consequently $a > 0$, and since $a + d < 0$, then we would get $d < 0$. Because $bc < ad$, we obtain $bc < 0$. If $b > 0$, then it is necessary that a is positive, c and d are negative, and D needs to be

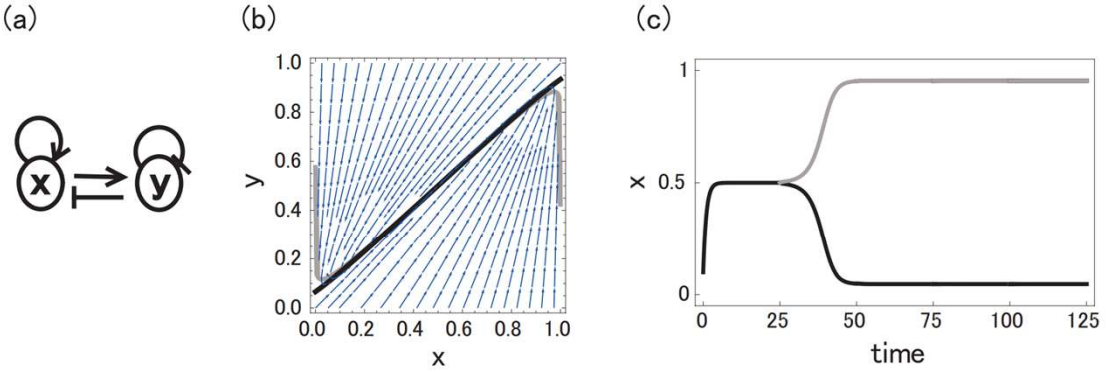


Figure 7.5 Differentiation process by Turing instability in a two-gene model (a) Example of a network exhibiting instability. (b) Nullcline of expression dynamics in one cell. (c) Time series of expression levels of gene x. As the number of cells increases through mitosis, two different stable states emerge from the cell-cell interactions. Assume that the value of J_{ij} takes the value

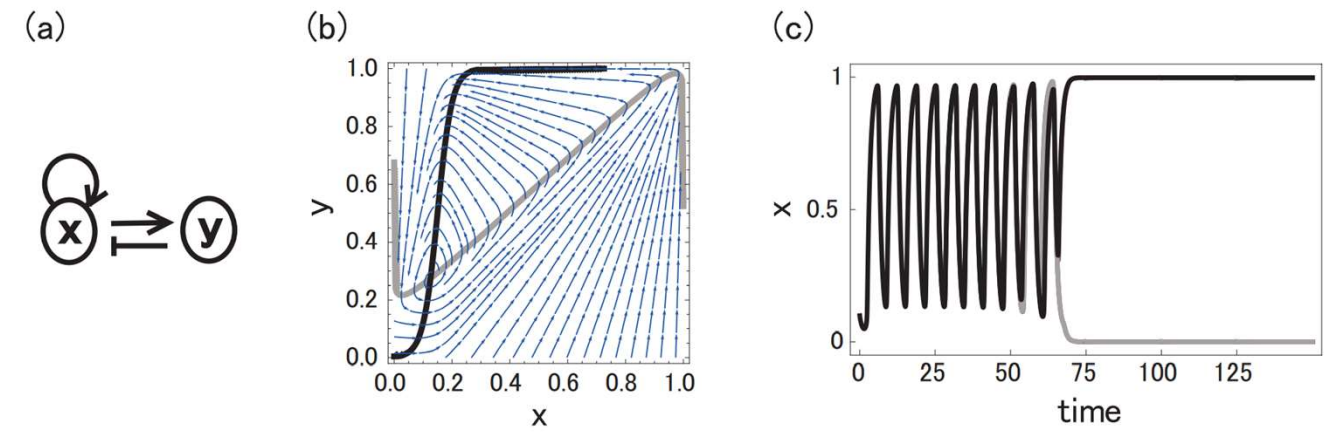


Figure 7.6 Differentiation from a limit cycle to fixed points in the two-gene model (a) Example of a network showing differentiation from a limit cycle to a fixed point. (b) Nullcline of expression dynamics in one cell. (c) Time series of the gene expression levels x. The parameters $(D, \theta_x, \theta_y) = (1, -0.1, 0.15)$ were used, and the other parameters were the same as those

Exercise 7.3: Following the case of class I, we draw a change in the nullclines and understand how two fixed points appear self-consistently.

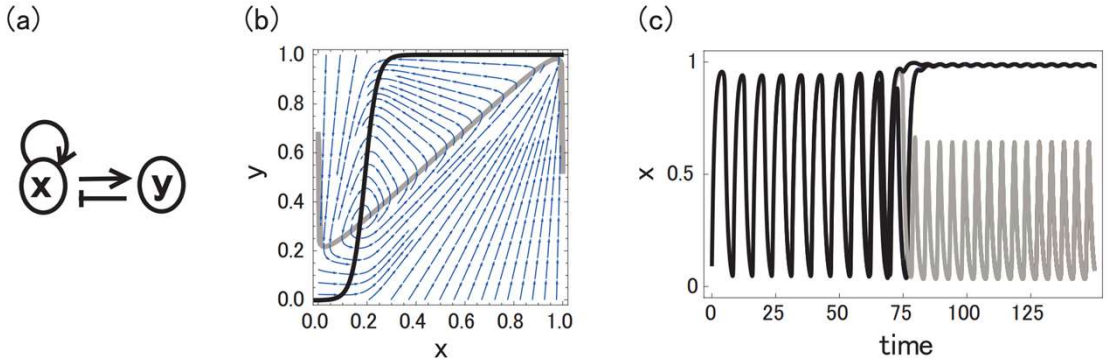


Figure 7.7 Differentiation processes that leave oscillating dynamics in the two-gene model. (a) An example network showing a differentiation process in which the oscillating dynamics remain. (b) Nullcline of expression dynamics in one cell. (c) Time series of the gene expression levels x. The parameters $(D, \theta_x, \theta_y) = (0.2, -0.1, 0.2)$ were used, and the other parameters were the same as those in Fig. 7.5

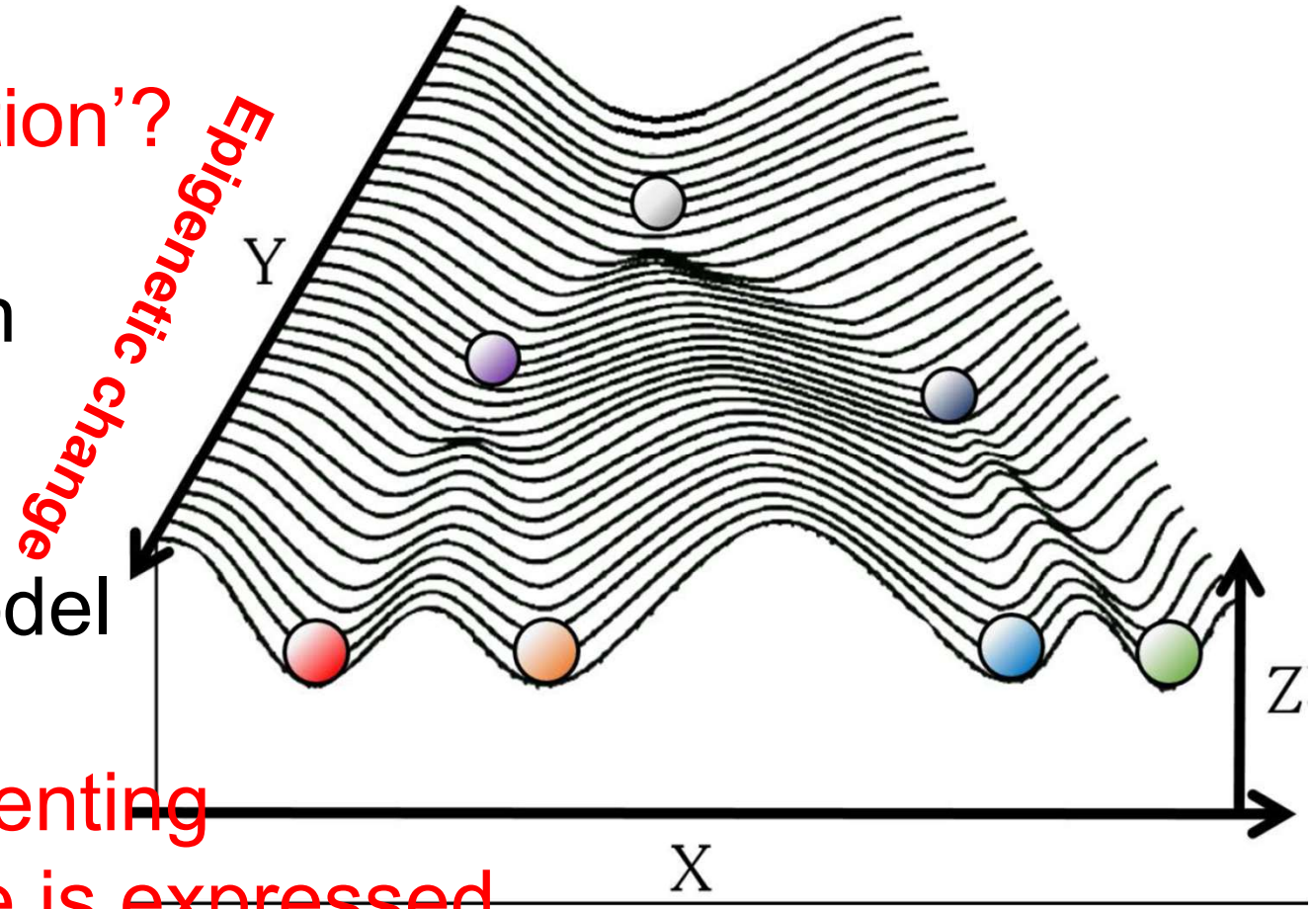
An Alternative Picture:
Y as the slow change in epigenetic modification?

(Matsushita, KK,2020, Phys Rev Res)

‘Epigenetic-Modification’?

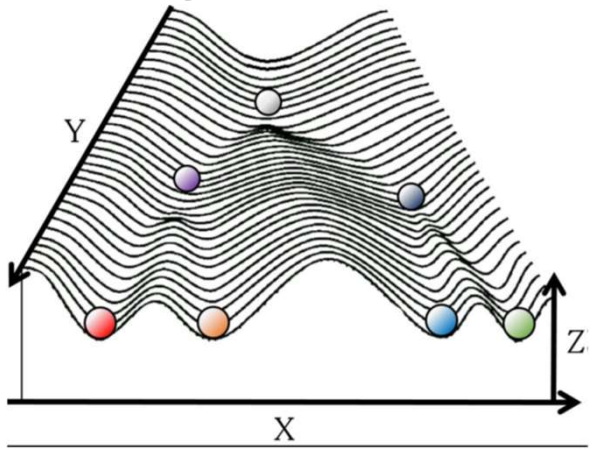
←Histon Modification
Methylation,,,,

“Coarse-grained” model
→
Slow variable representing feasibility that a gene is expressed (by chromatin change)



Y as slow epigenetic modification change

Question:epigenetic landscape of *Waddington's type* emerges?



1)Landscape(depth and positions of valleys) is robust to perturbations/initial conditions

1')Multilevel-robustness?: The number ratio of each cell type is robust

2)Homeorhesis?: stability of branching process and timing

3)Hierarchical branching？

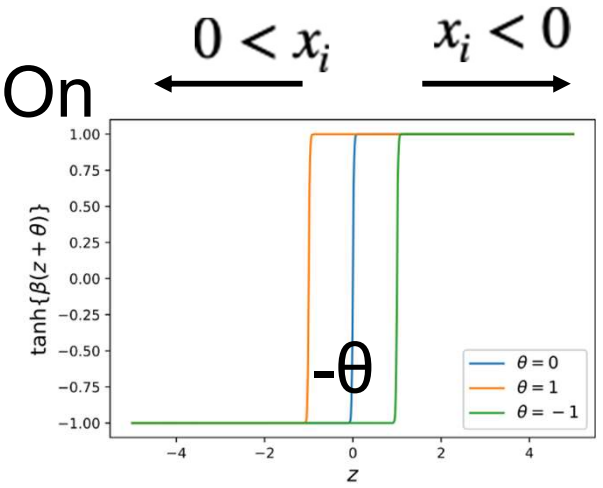
Cell model (GRN + epigenetic modification)

x_i $(i = 1,2,\ldots,N)$ Gene expression

x_i + expressed (max 1) On

- Repressed Off

$$\frac{dx_i}{dt} = \tanh\beta\left(\sum J_{ij}x_j + \theta_i\right) - x_i$$

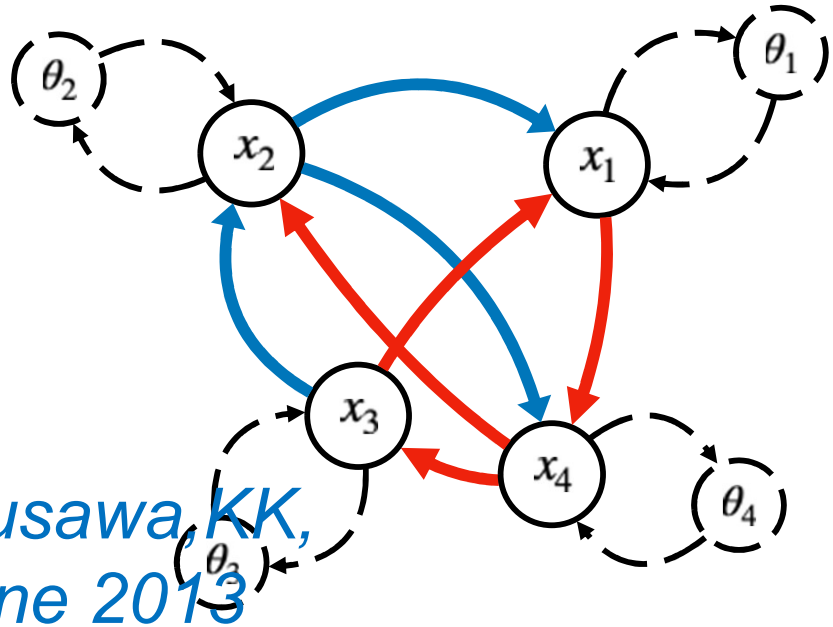


$-\theta_i$ Threshold for expression

θ_i → Variable for epigenetic modification

Gene regulatory network (GRN)
: regulation matrix

J_{ij} + : activate
:- inhibit



+Positive feedback from Expression

$$\frac{d\theta_i}{dt} = v(ax_i - \theta_i)$$

If expressed, easier to be expressed: If repressed, harder to be expressed

Cf: Furusawa, KK, PLoS One 2013

a: strength of feedback

***v:* rate of epigenetic feedback**

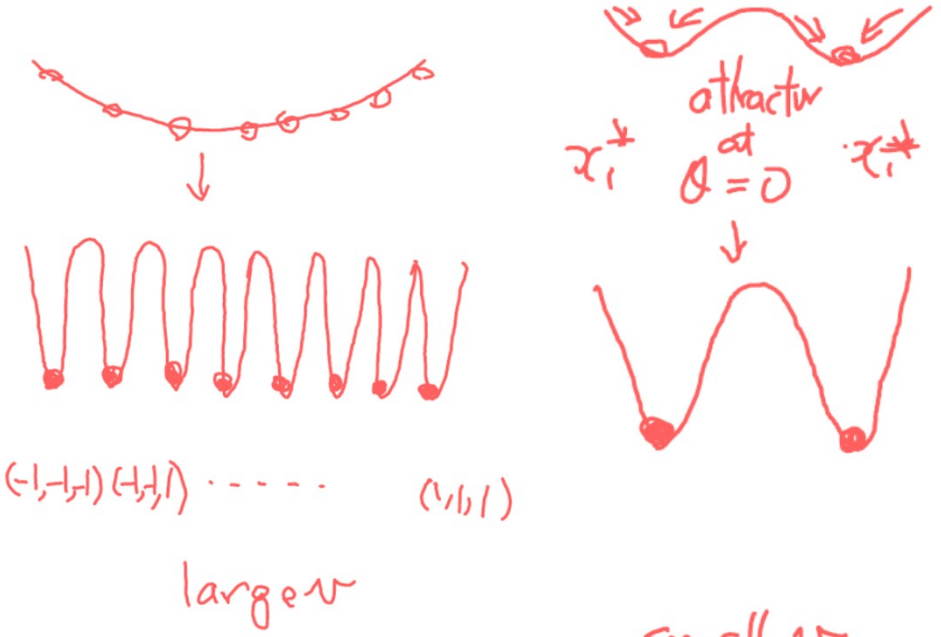
Fixed point analysis

$$\frac{dx_i}{dt} = \tanh\beta\left(\sum J_{ij}x_j + \theta_i\right) - x_i = 0$$

$$\frac{d\theta_i}{dt} = \nu(ax_i - \theta_i) = 0$$

x_i^* Fixed point satisfies

$$\tanh\beta\left(\sum J_{ij}x_j^* + ax_i^*\right) - x_i^* = 0$$



For large *a*, *all* $x_i = \pm 1$ are fixed point attractors

Q: Initially θi is set to 0 (no epigenetic-modif.)

Then, which attractors are selected?

$\nu \to \infty$ (or large)

All of 2^N states reached, just following initial expression x

$\nu \to 0$ (or small) **←Slow Epigenetic Modification case**

Only a part of initial fixed-pt attractor of x (with θ＝0)

is stabilized

If attractors of initial (θi=0) dynamics are fixed points →they are fixed with θ depending on the initial condition

Multiple types possible, but

→1)For each basin, only one type of fixed pt is generated → no robustness against changes in the initial expression

small v
(adiabatic)

→ 1')number ratio of each type non-robust (i.e., no *homeorhesis*)

→2)No robustness in time-course,

→3)No hierarchical splitting in time

→ Cannot support Waddington landscape

When initially oscillatory state (limit-cycle for θi＝０）

First, limit-cycle attractors are converged
Then with the change in θi (i.e. by slow epigenetics),
 a few fixed-point attractors are generated.
Generally observed for most networks with large N (say ≳10)

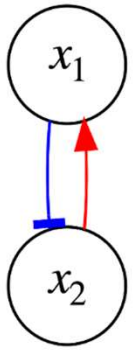
will show→

1)Robustness against changes in the initial expression
1’) number ratio of each type is robust
2) Robustness in time-course, number ratio of each type
(i.e., no *homeorhesis*)
3) Hierarchical splitting in time

←Interference between fast oscillation & slow fixation

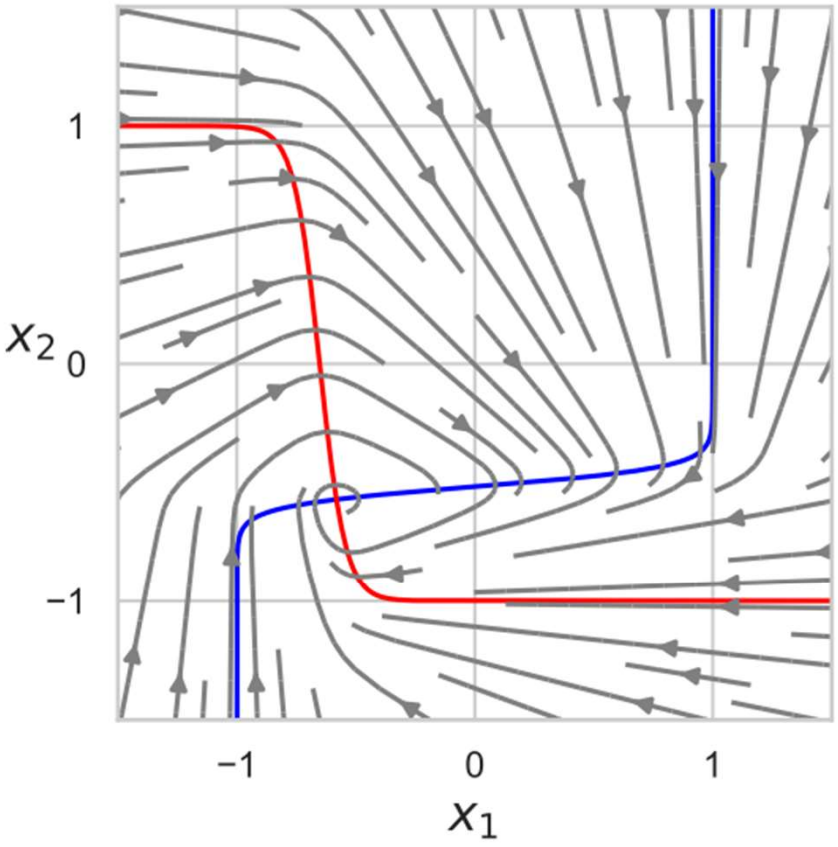
1)2) Minimal model; 2-gene :

Limit-cycle globally reached, then fixation with θ change

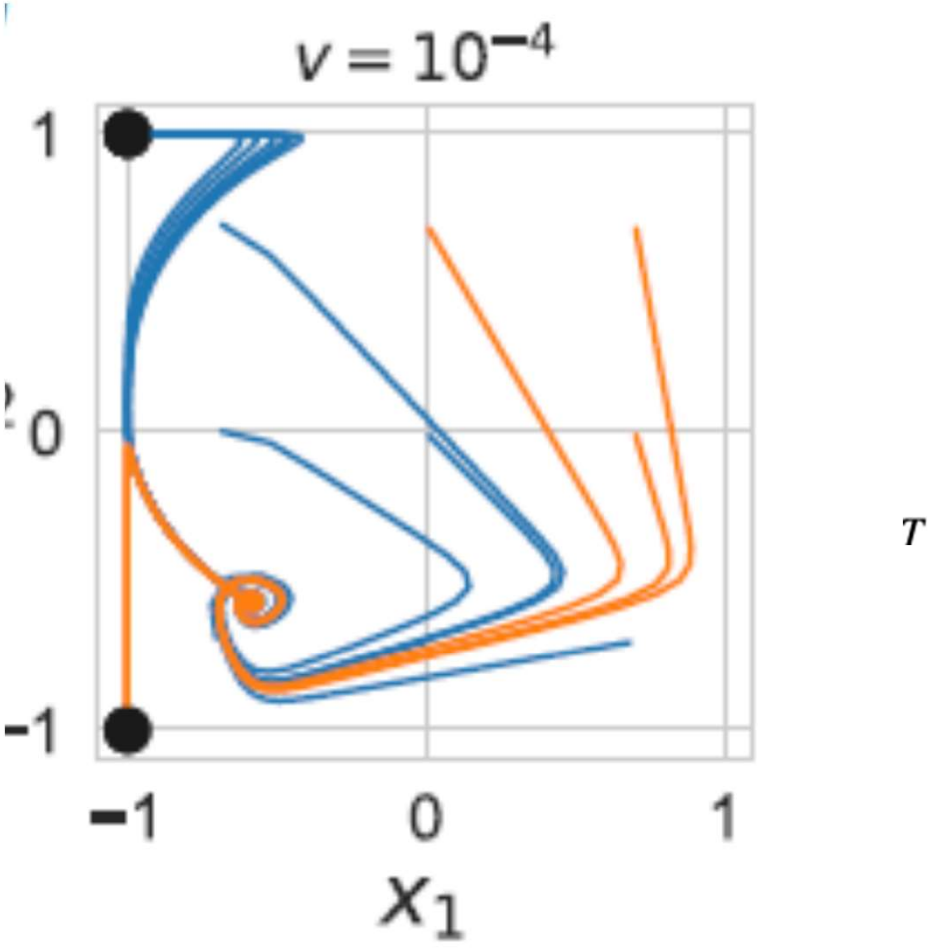


$$\frac{dx_1}{dt} = \tanh\beta(J_{12}x_2 + c_1 + \theta_1) - x_1, \quad \frac{d\theta_1}{dt} = \nu(ax_1 - \theta_1)$$

$$\frac{dx_2}{dt} = \tanh\beta(J_{21}x_1 + c_2 + \theta_2) - x_2, \quad \frac{d\theta_2}{dt} = \nu(ax_2 - \theta_2)$$



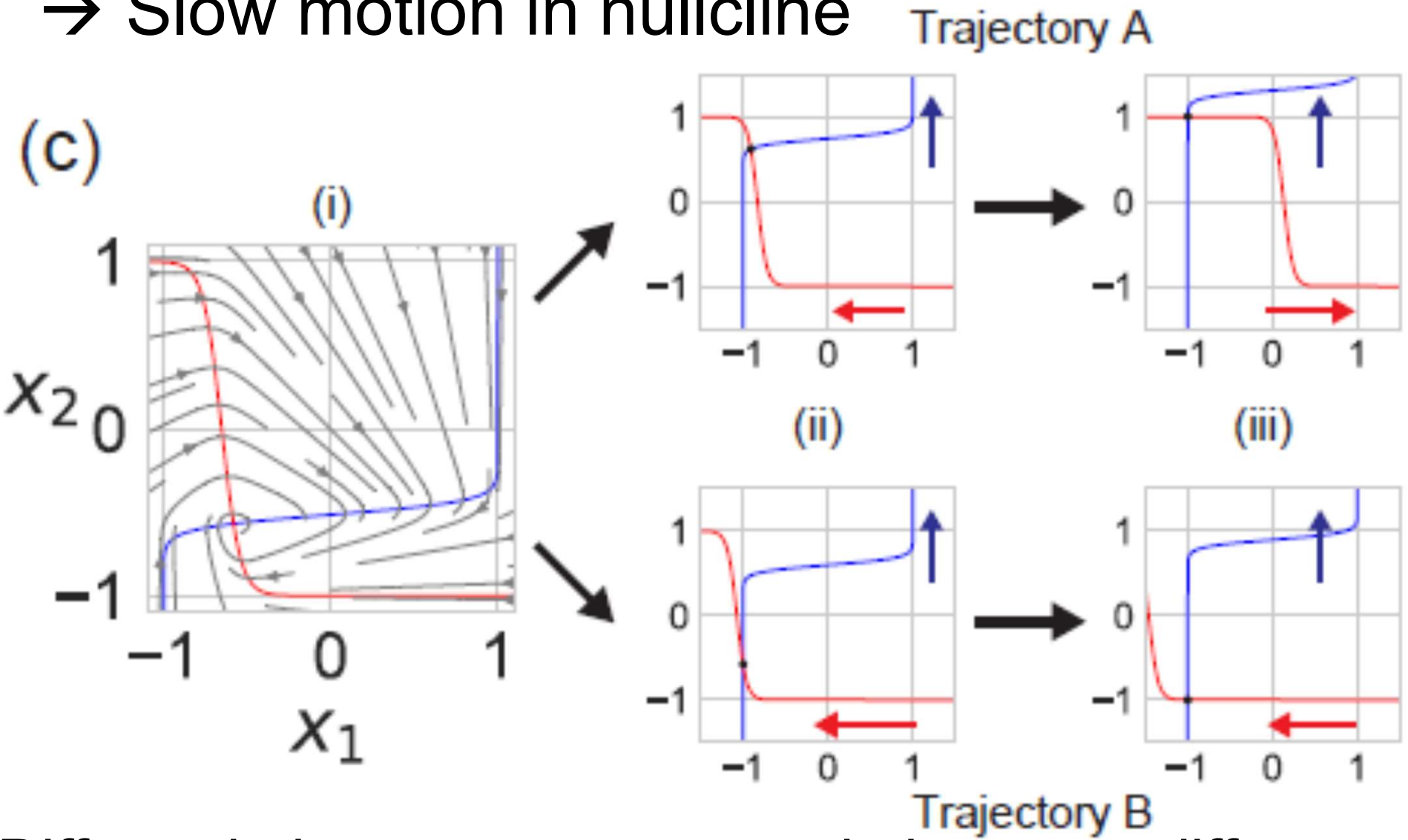
Dynamics for fixed θ = **0**



$J_{12} = 0.31, J_{21} = -0.23 \quad c_1 = 0.16, c_2 = -0.15$

Slow change in θi (by reinforcement)

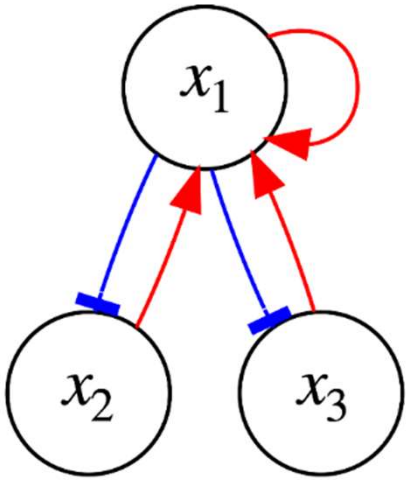
→ Slow motion in nullcline

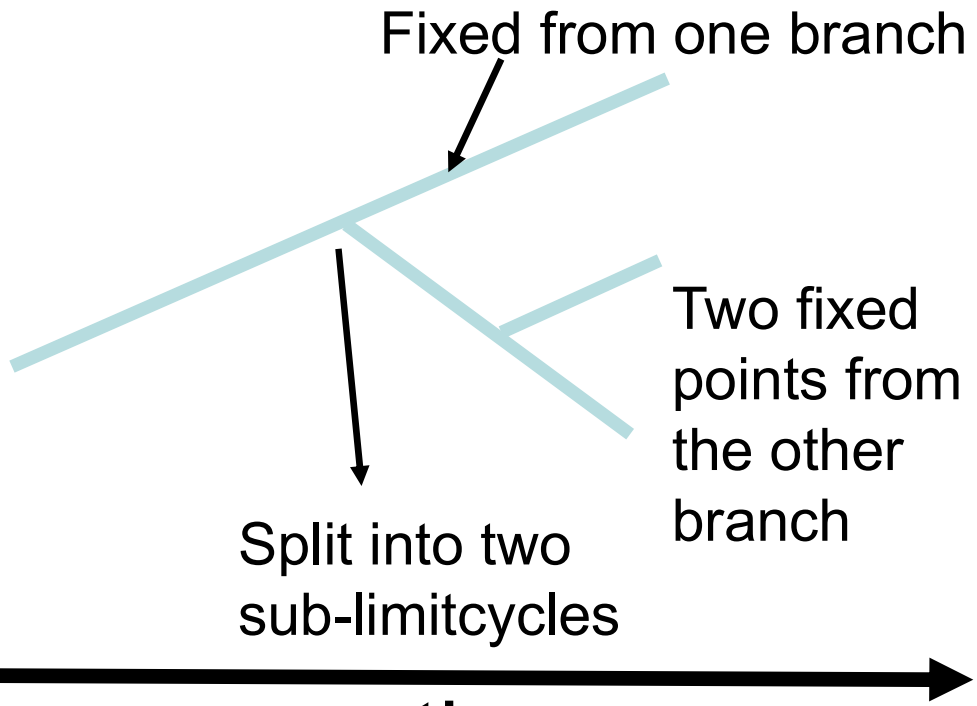


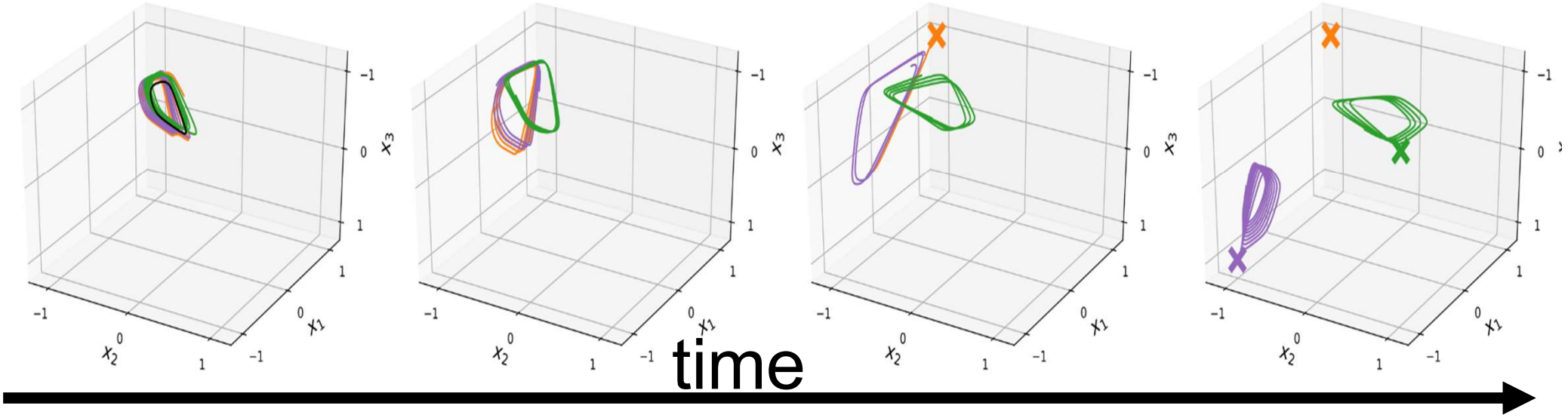
Differentiation occurs at same timing, over different initial conditions. In their vicinity, different fixed points are generated, with certain proportion →

2)3) Hierarchical splitting with homeorhesis

Simplest example N=3







Branching occurs at the same timing (indep't of initial conditions)

Hierarchy of trajectory

☑ Hierarchical Branch

Traveling over phase space with oscillation

→ Hierarchical trajectory separation

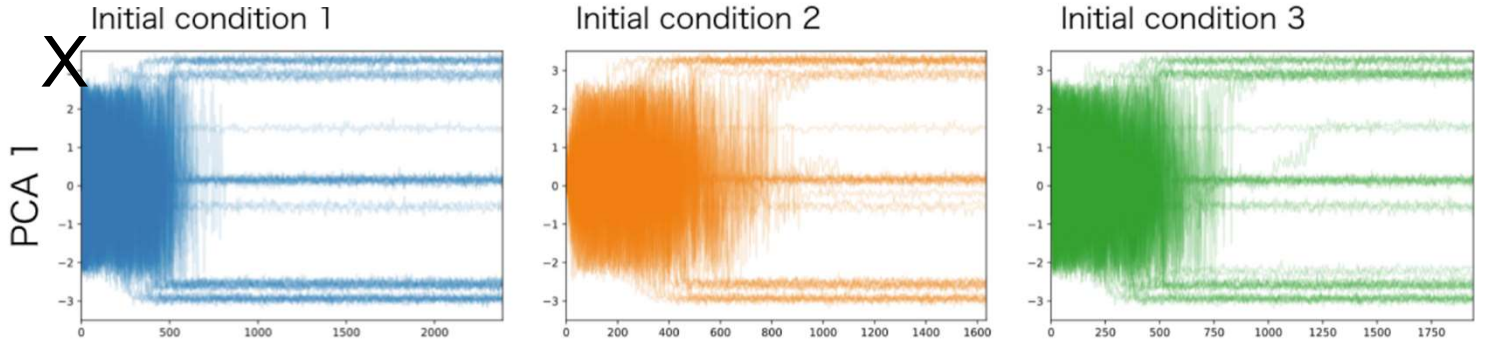
Hierarchical attractor-generation from limit-cycle (HAGL) satisfies Homeorhesis

N=10 genes

500 cells with different initial conditions: ***X (PCA from xi)***

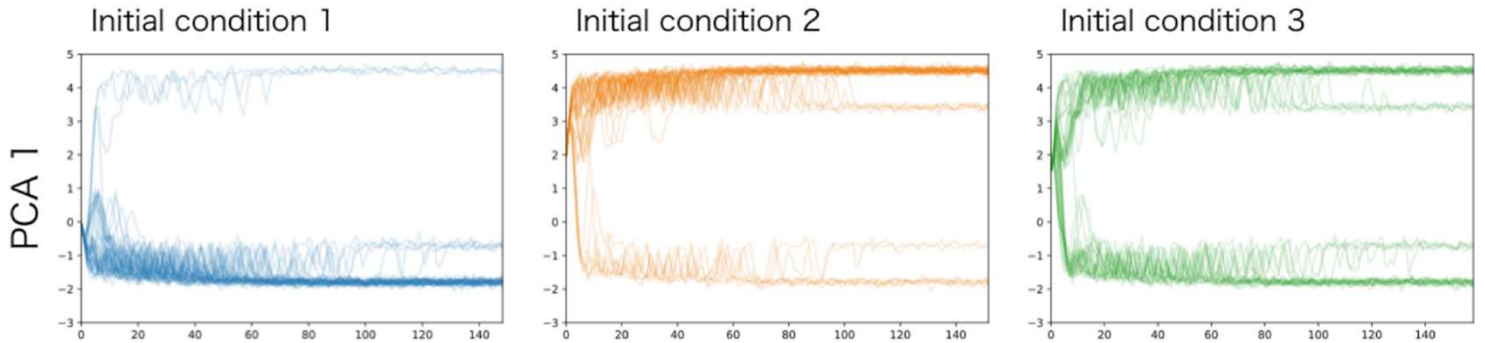
mpling many orbits from 3 initial distributions + noise

HAGL



HAGL Distribution, time-course robust

Fixed point case



Not robust

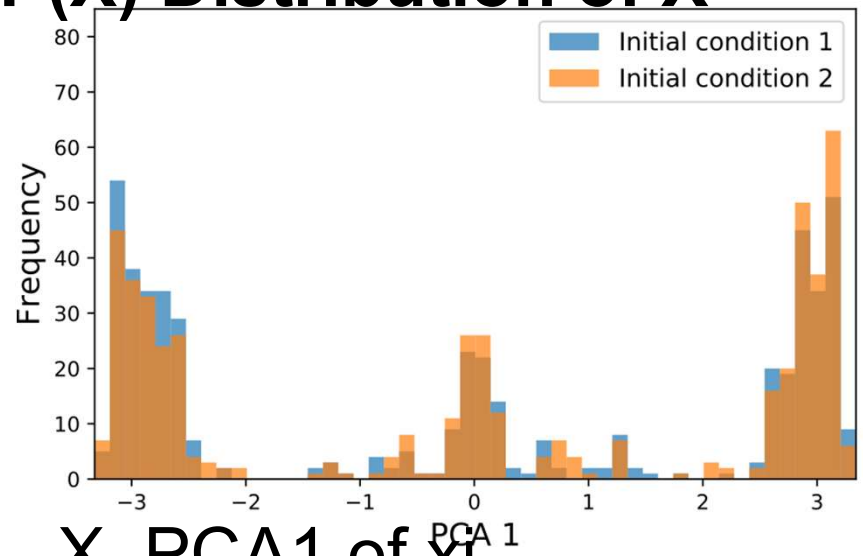
Once converged to limit-cycle, then distributed

→ Initial randomness or by perturbation is smeared out

Homeorhesis -- distribution of final P(X)(under noise)
– indep of initial conditions

HAGL, from oscillatory state

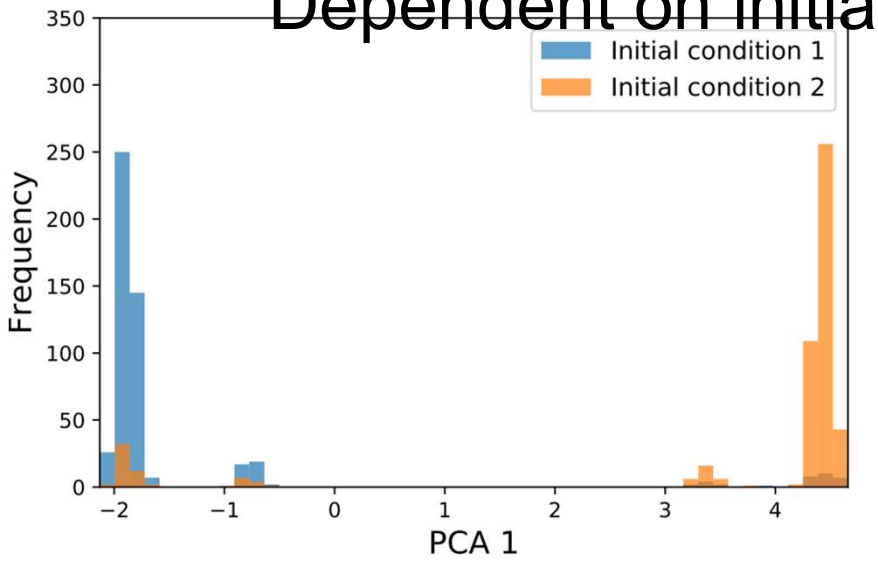
P(X) Distribution of X



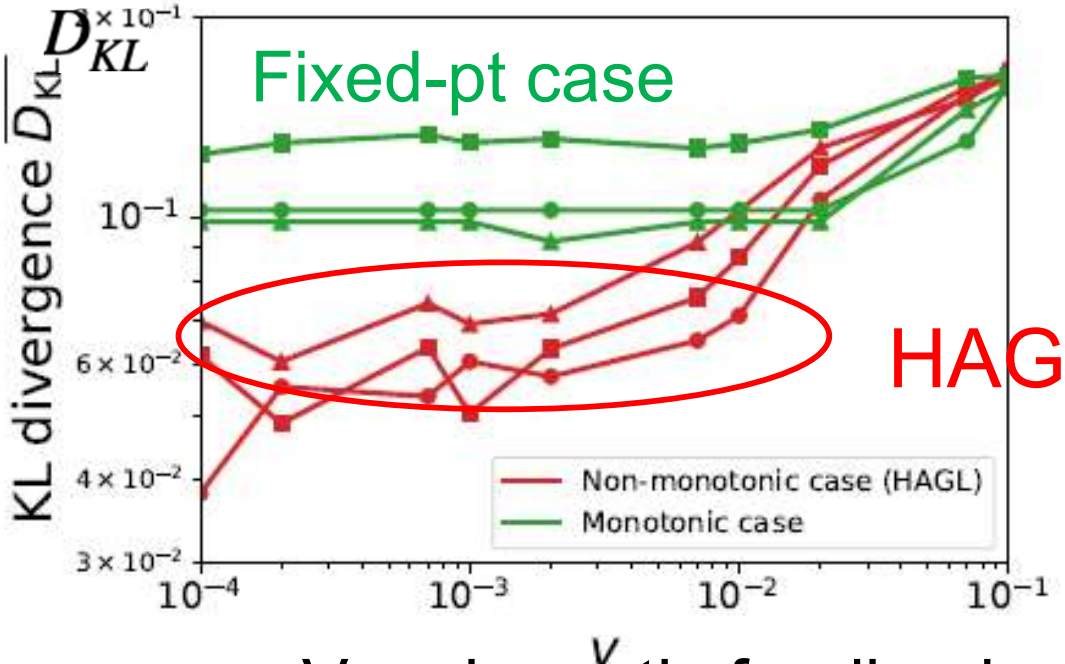
X, PCA1 of Xi

Fixed Pt case

Dependent on initial



Difference in distributions over many Initial conditions --
Measured by KL divergence



V: epigenetic feedback ra

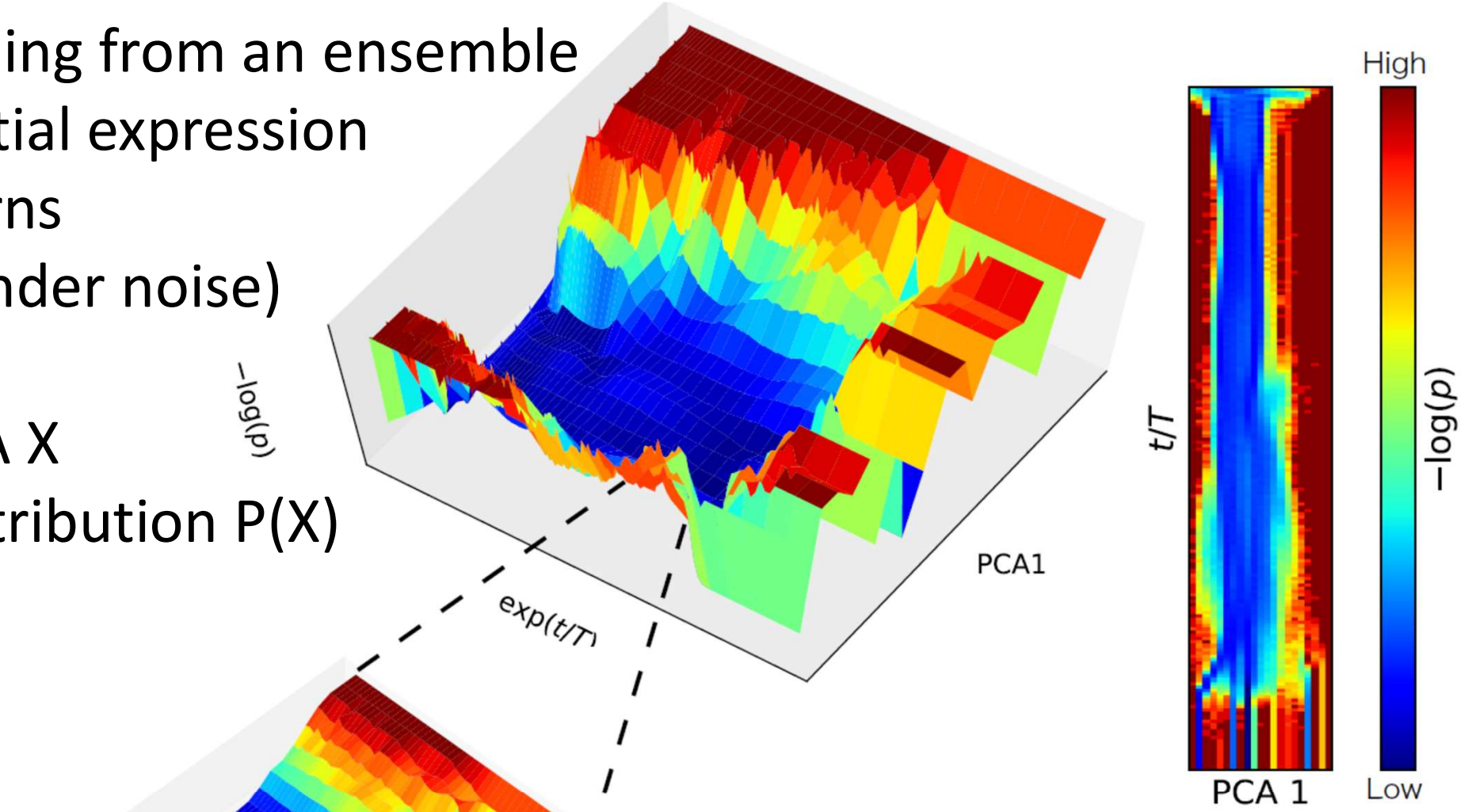
Epigenetic Landscape generated from Limit-cycle state

☑ hierarchical branching ☑homeorhesis

Sampling from an ensemble of initial expression patterns (or under noise)

→PCA X

→Distribution P(X)



X- axis : PCA 1 (gene expression space)

Y- axis: Time for slow epigenetic change

Z- axis : log(1/P(X)): P prob distrb

II. Reprogramming

induced Pluripotent Stem cells Takahashi&Yamanaka 2006

Developmental potential

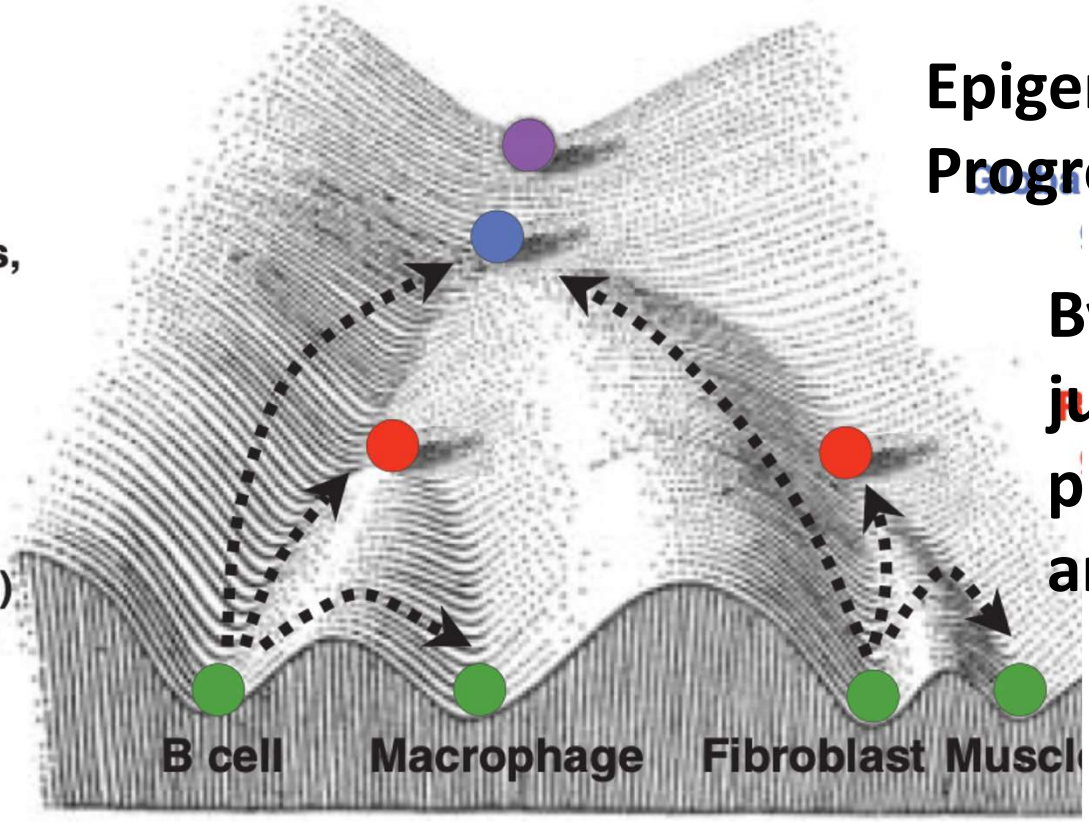
Totipotent
Zygote

Pluripotent
ICM/ES cells, EG cells, EC cells, mGS cells iPS cells

Multipotent
Adult stem cells (partially reprogrammed cells?)

Unipotent
Differentiated cell types

Reverse irreversible differentiation!



Epigenetic Modification Progresses with time

By overexpressing just 4 genes, initial pluripotent states are recovered

How cells can return to “unstable” pluripotent state?

How only four genes can erase most of epigenetic memories?

Questions

1. How can the pluripotent state be regained only by overexpression of few genes (without operation of epigenetic modification)?
2. How can such common simple operation bring different cells back to the same pluripotent state?
3. How can reprogramming robustly make the cells head toward such an “unstable” (saddle?) state?

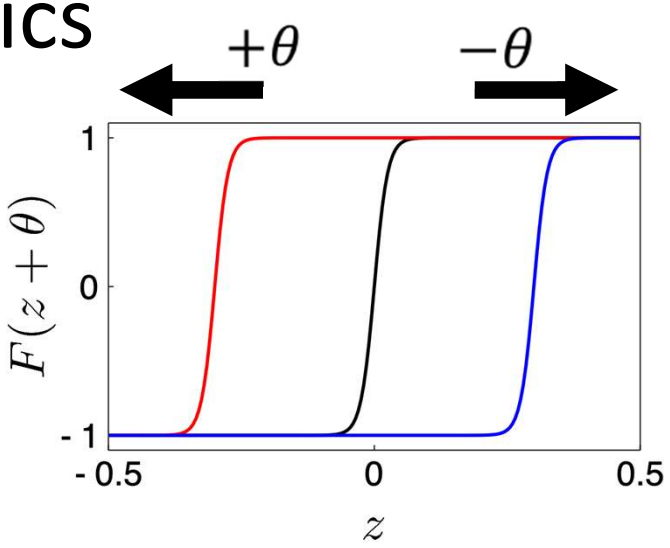
By Oscillations in gene expression & Slow epigenetic modification

Differential equations with gene regulatory network + epigenetics

x_i: i -th gene expression level

θ_i: i -th epigenetic modification level

$$\frac{dx_i}{dt} = F\left(\sum_j J_{ij}x_j + \theta_i + I_i(t)\right) - x_i$$



Positive θ_i value
→ feasible expression
(otherwize)

θ_i threshold

Reprogramming manipulation
(external input to gene expression)

$$\frac{d\theta_i}{dt} = \frac{1}{\tau}(x_i - \theta_i)$$

τ : timescale of epigenetic modification ($\tau \gg 1$)

τ=1/v

Epigenetic modification dynamics
→ reinforcement of gene expression

(expressed genes become more expressed)

Reprogramming Works in our Model!

Take any of differentiated states (x~θ fixed to ~1 or ~0)

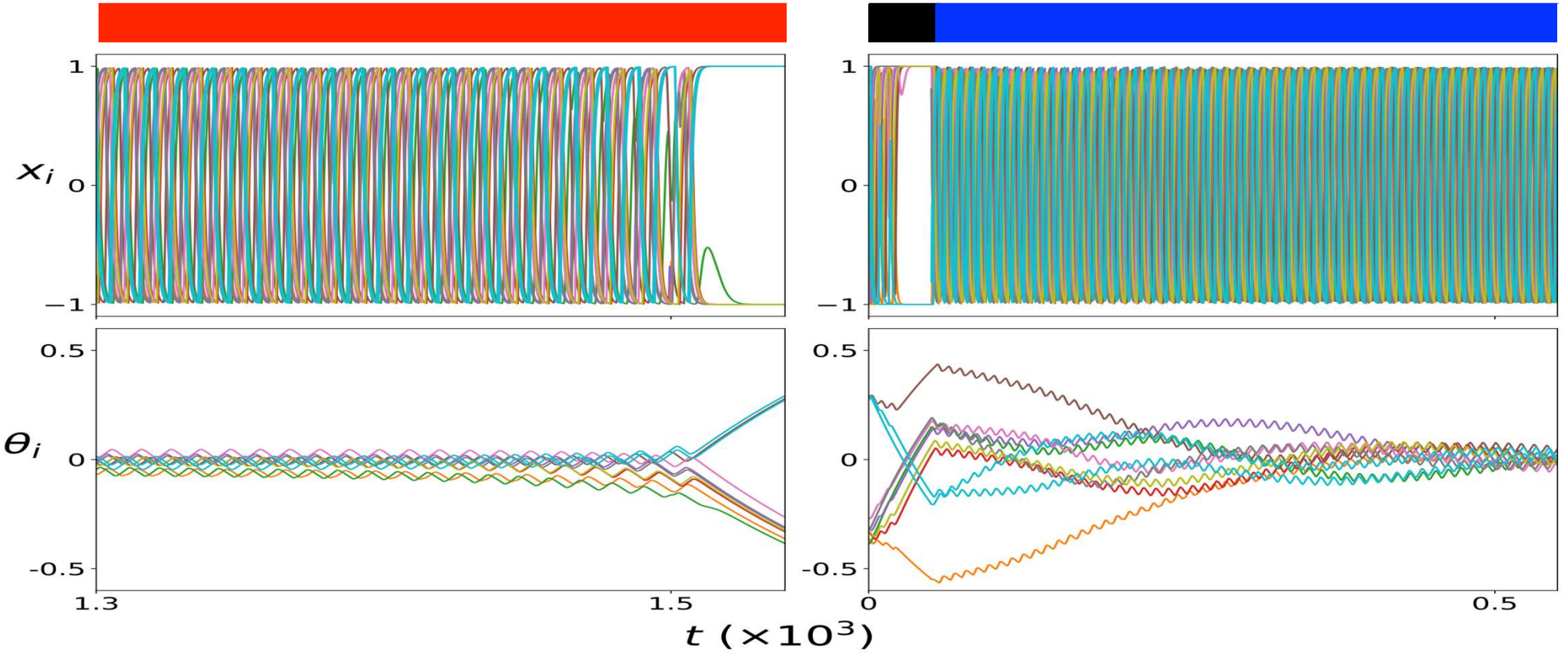
Apply inputs Ii to few genes i for some time span

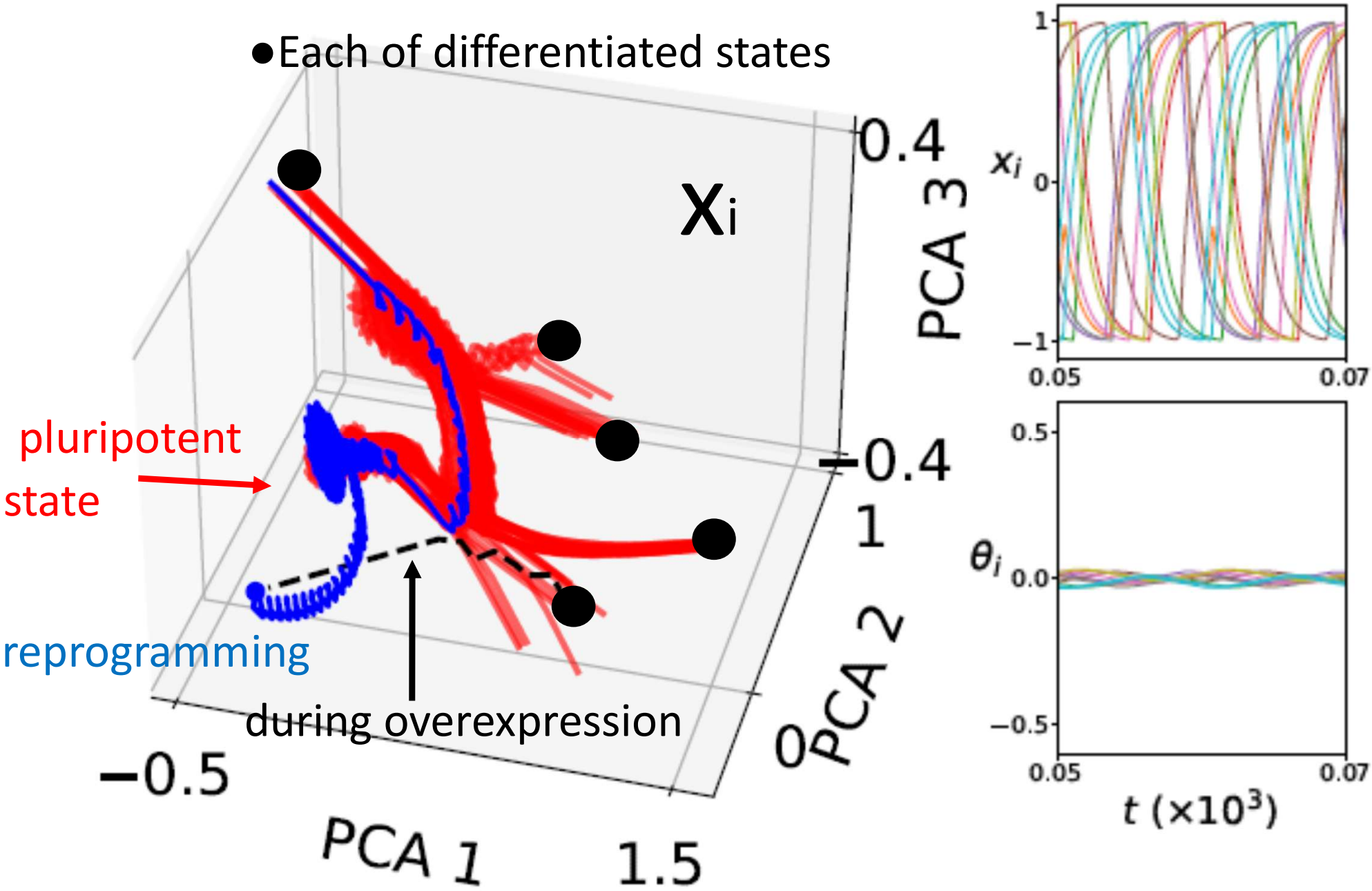
→ x regains oscillation, θ approaches 0

Regains pluripotency! →differentiation progresses

An example with N=10

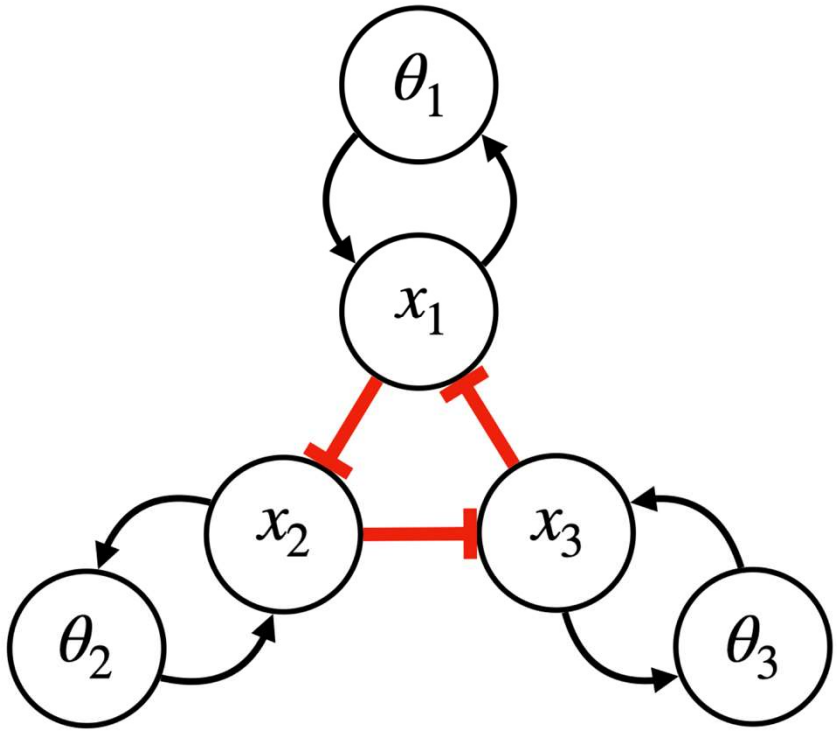
Differentiation → overexpression just 3 → stop manipulation

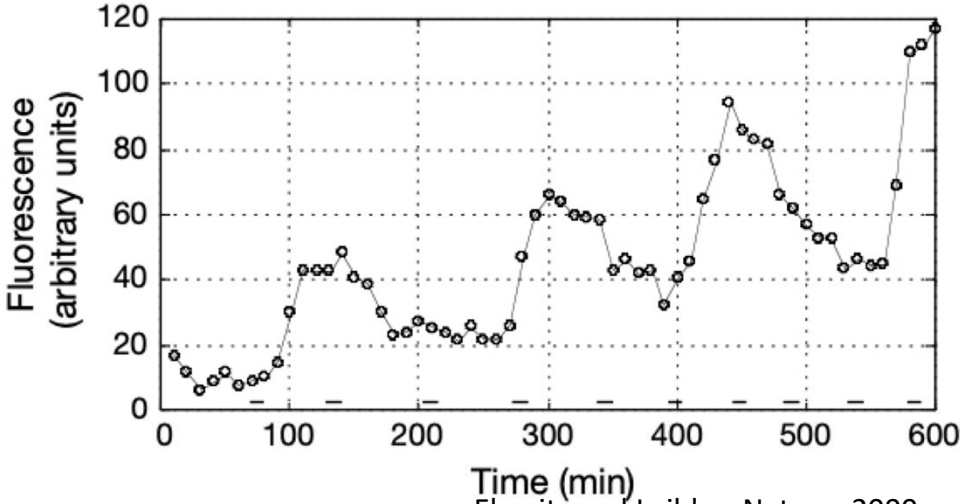



●Each of differentiated states
Xi
pluripotent
state
reprogramming
during overexpression
PCA 1
PCA 2
PCA 3
−0.5
0
1.5
1
−0.4
0.4
x_i
θ_i
1
0
−1
0.5
0.0
−0.5
0.05
0.07
t (×10³)

How possible?
Simple Example: To understand the Mechanism
Repressilator genetic circuit

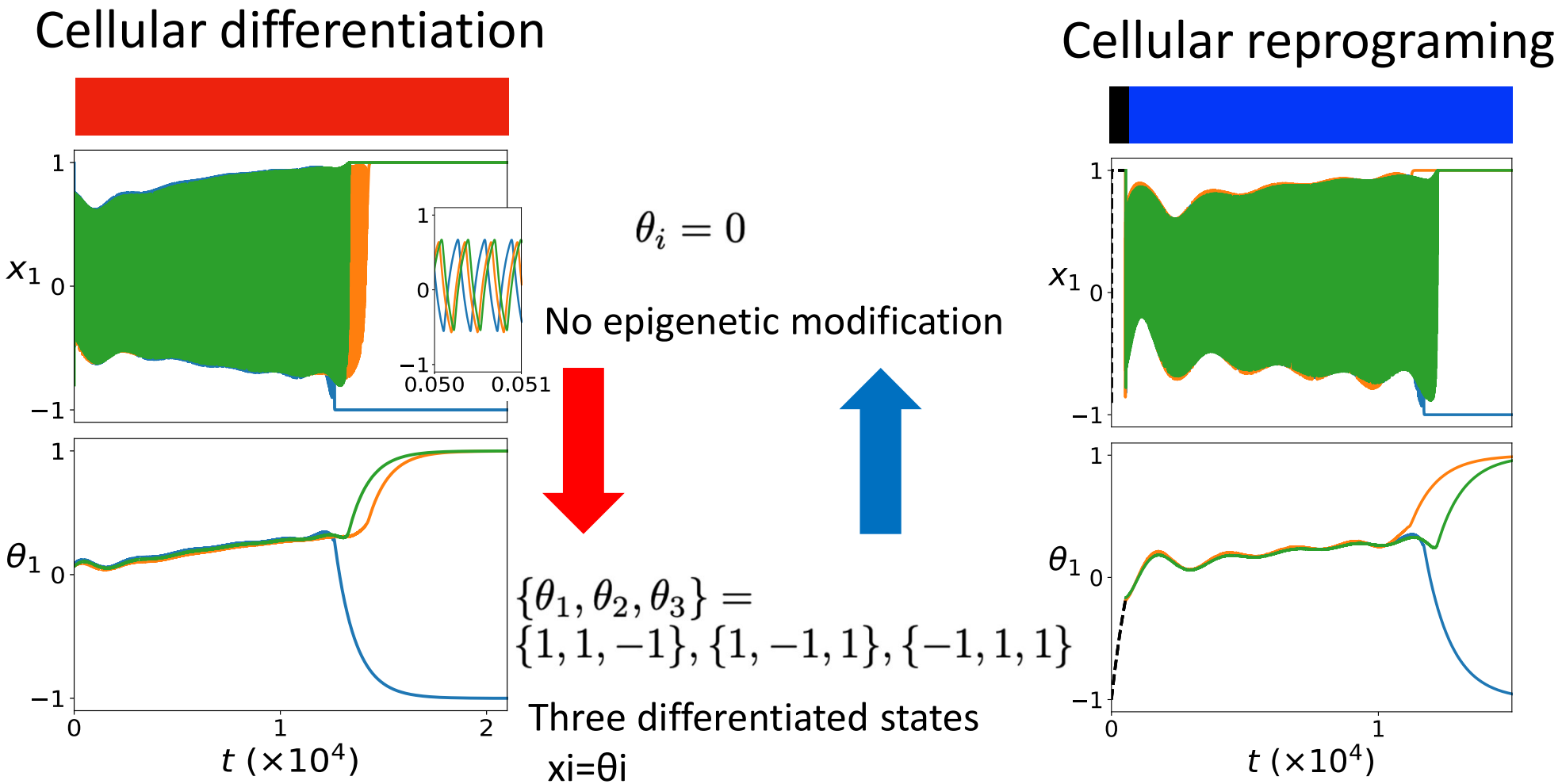
Minimal oscillatory genetic circuit





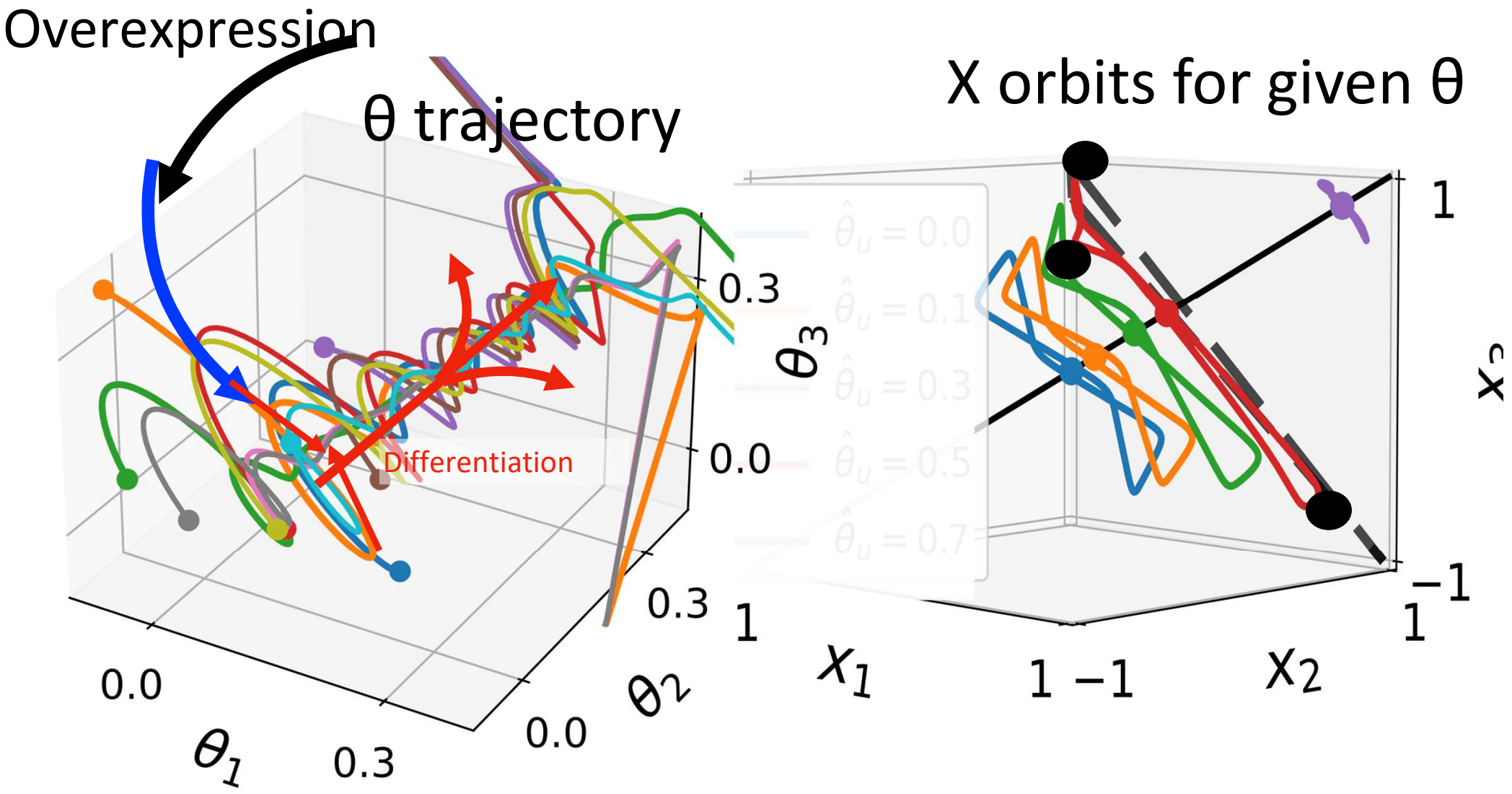
Elowitz and Leibler, Nature, 2000

Both cellular differentiation and reprogramming are achieved by oscillation and epigenetics



Dynamics after reprogramming manipulation

→ x-oscillation leads to attractive pathway towards θ~0



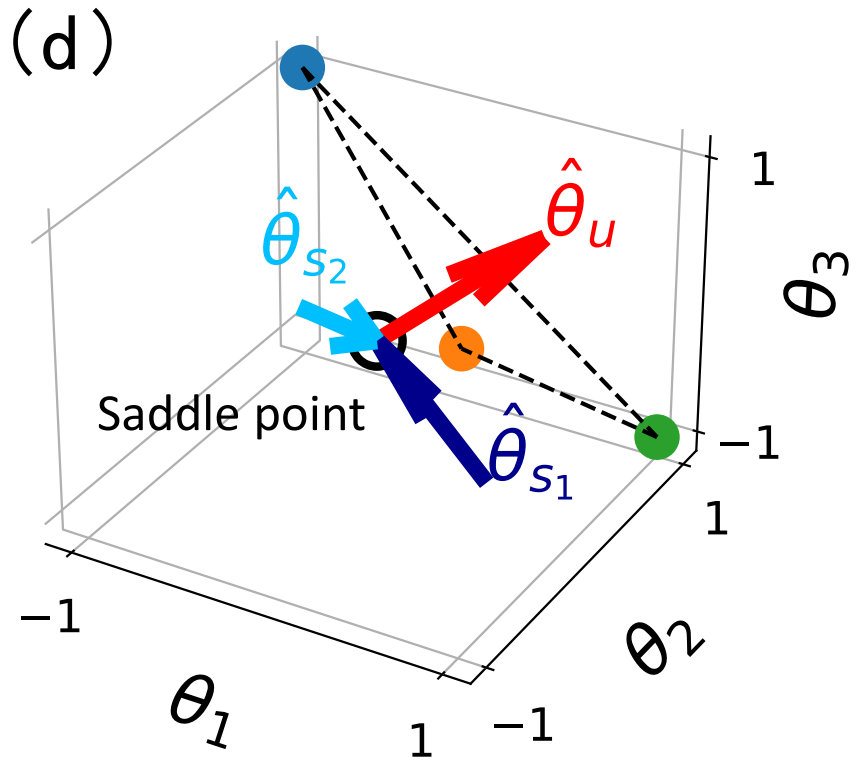
· Return to “unstable” state from any of fixed points (erasing epigenetic memories) --how?

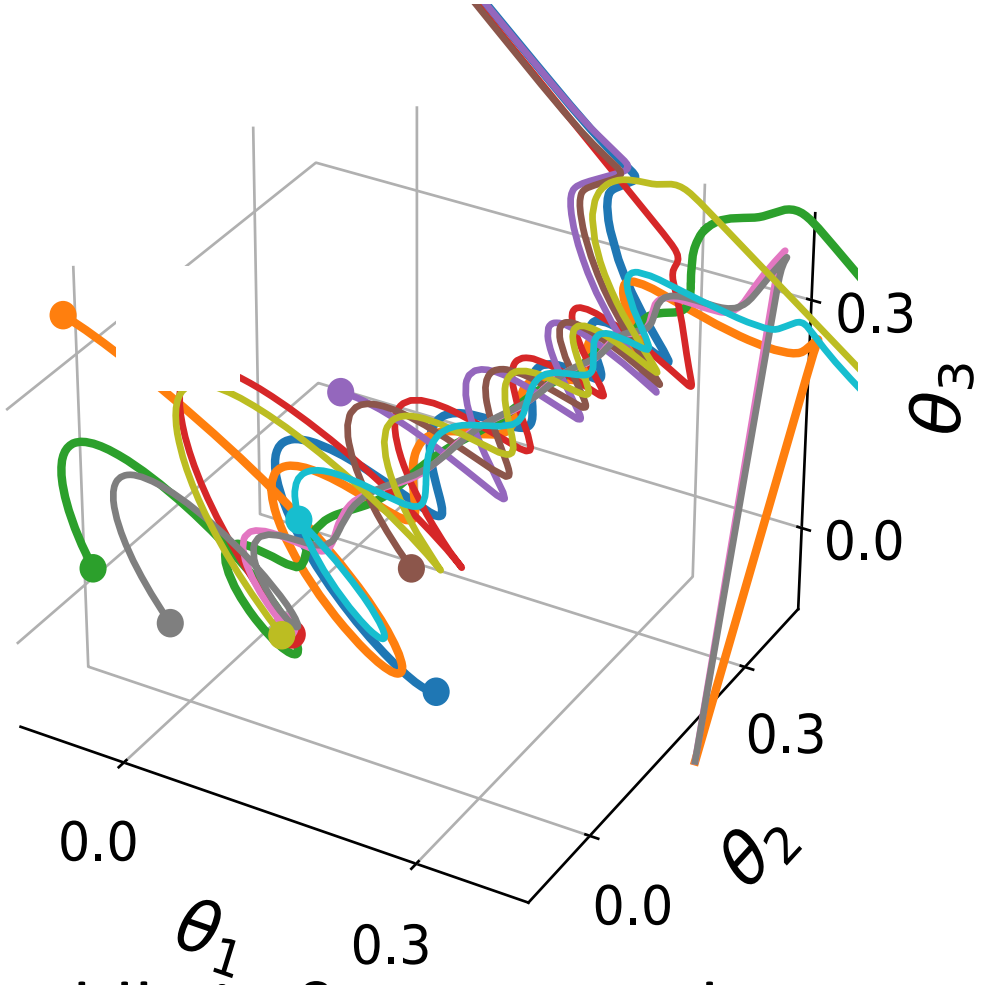
Epigenetic Dynamics:

Slow-θ limit: analyzed by timeaverage x dynamics

$$\frac{d\theta_i}{dt} = \bar{x}_i(\theta_i) - \theta_i$$

→ θ＝O saddle

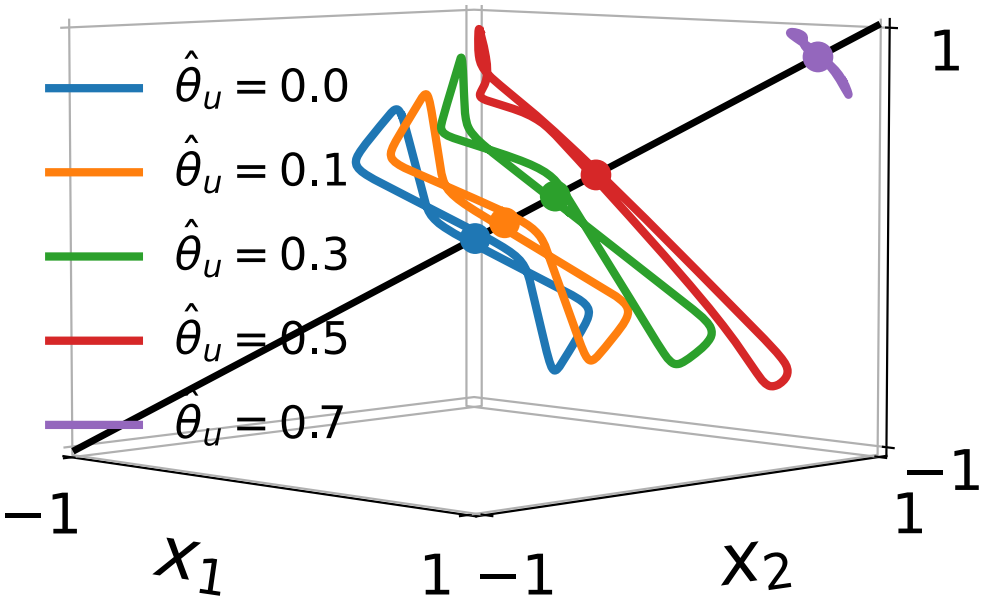


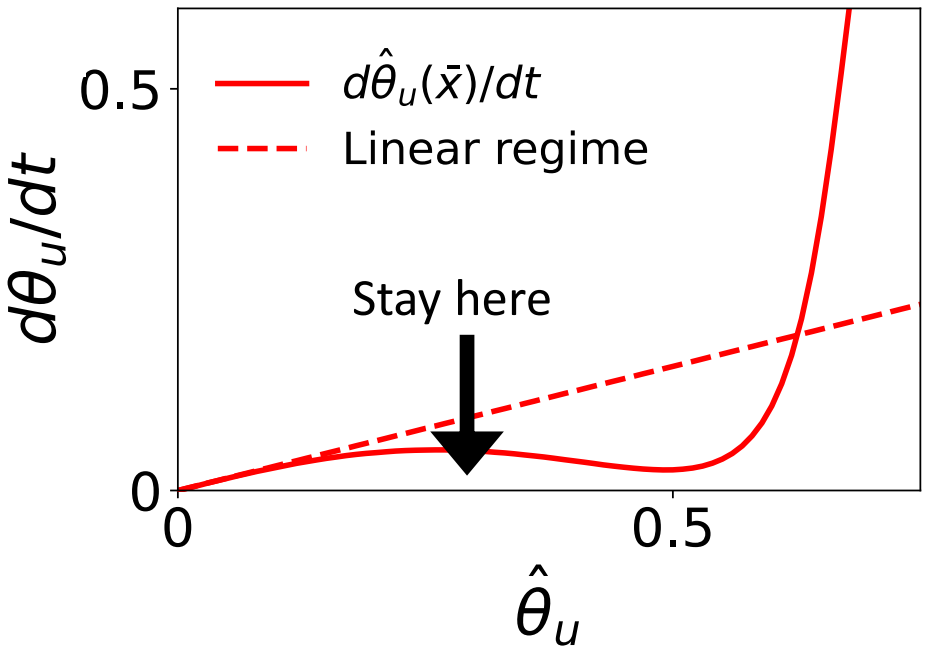


Attracted globally towards the saddle in θ-space, and move slowly along its unstable manifold

Due to oscillation in x, instability along unstable-manifold in θ is suppressed

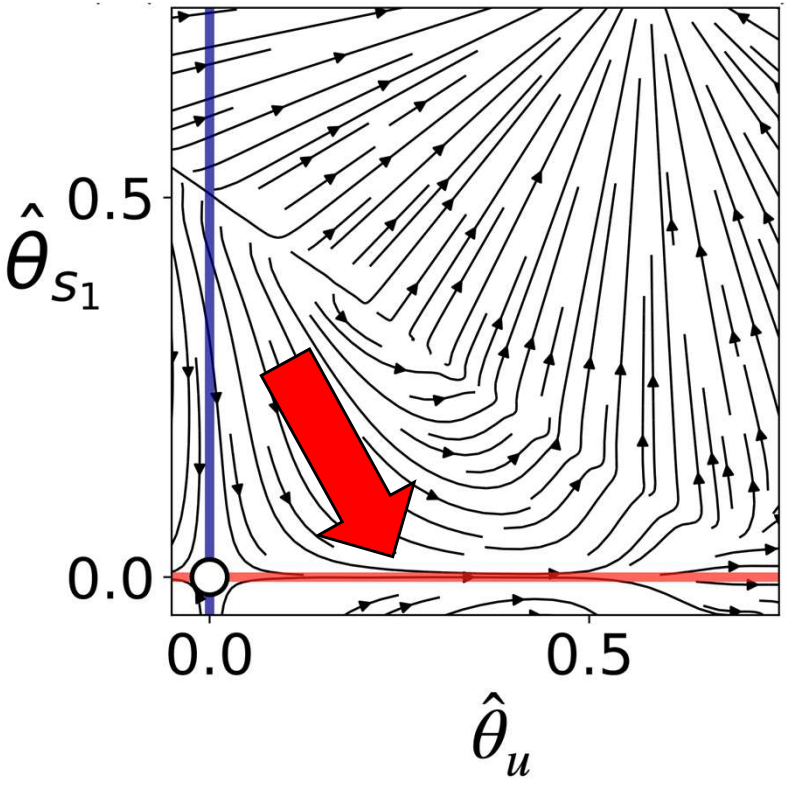
$$\frac{d\theta_i}{dt} = \bar{x}_i(\theta_i) - \theta_i$$

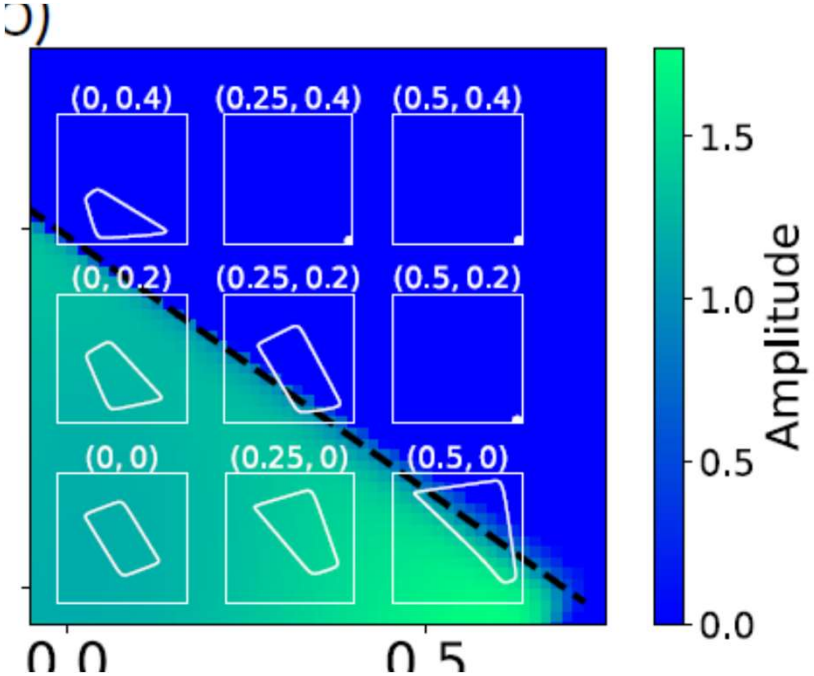




→ Stay in the vicinity of “unstable”manfiold over a long time span

Epigenetic states from different cell types globally converge towards θu and change slowly along it

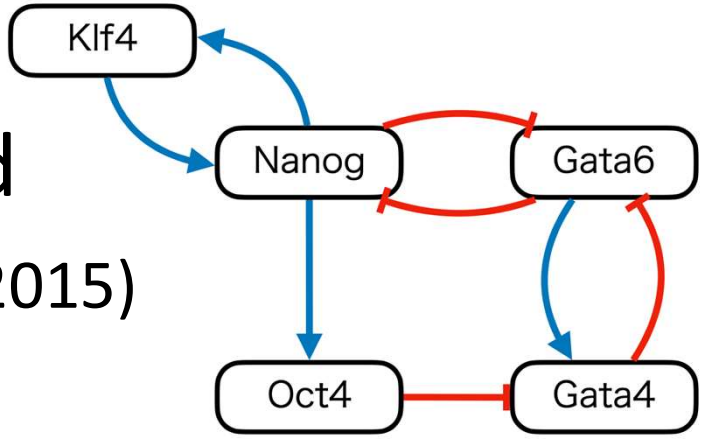




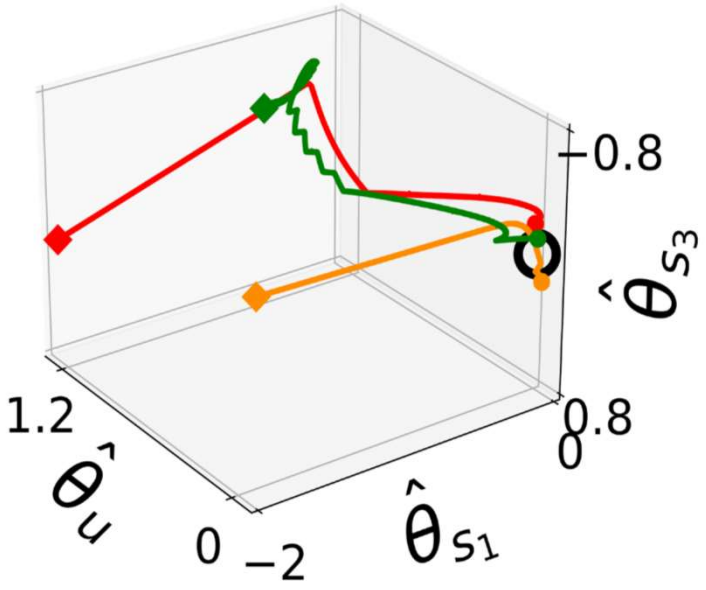
$$\frac{d\theta_i}{dt} = \bar{x}_i(\theta_i) - \theta_i$$

Same mechanism works universally

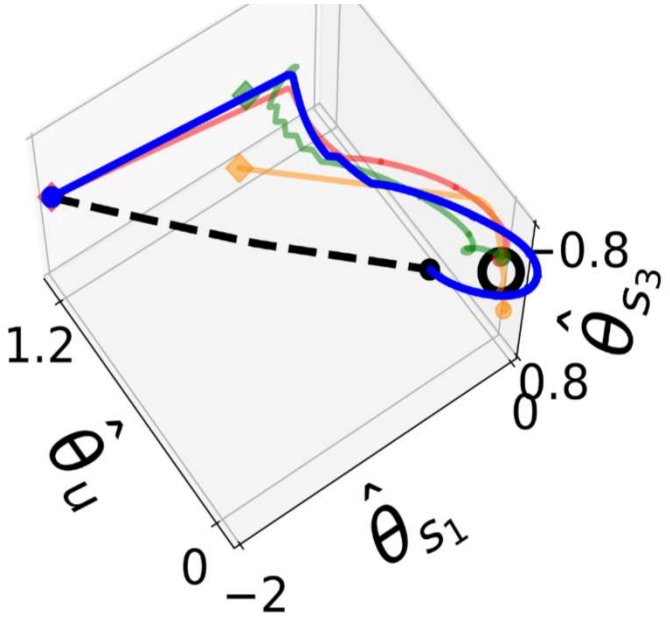
Gene regulatory network extracted from ES cells (Miyamoto et al., 2015)



Differentiation



Reprogramming



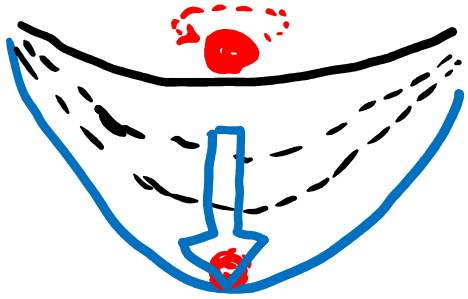
Reprogramming is completed after overexpression of

$Oct4, Nanog, Klf \quad (Sox2)$

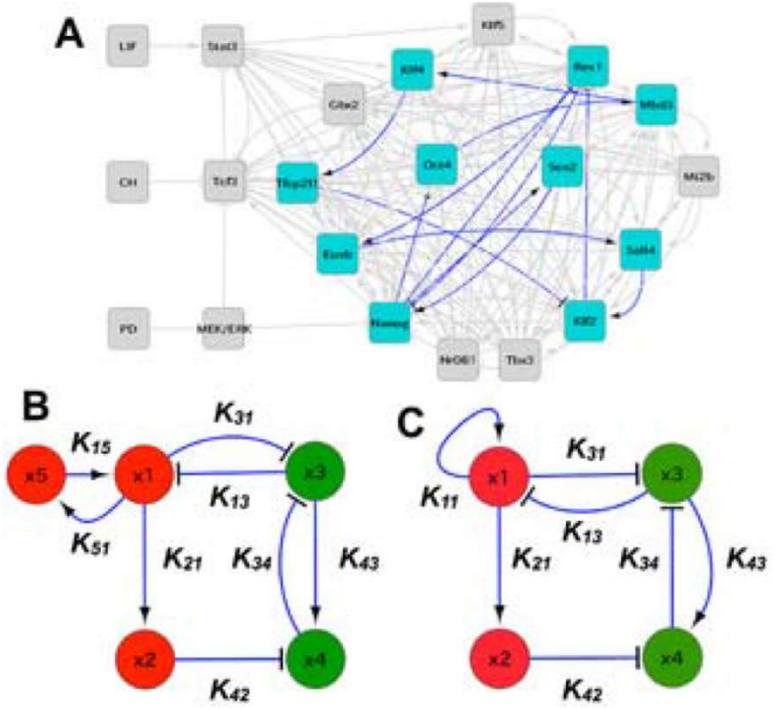
Combining the interaction-induced differentiation + epigeneitc change →Irreversible Fixation of differentiation

Miyamoto,Furusawa,KK PLosCB 2015

protein composition change (expression level) → 'epigenetic' change



"*'as if ball deepens the valley of potential*"



$$\frac{dp_i^k(t)}{dt} = f(\sum_j W_{ij}p_j^k, \theta_i^k)) - p_i^k + D_i(\overline{p_i} - p_i^k)$$

$D_i \neq 0$ only for few permeable components

$$f(x, \theta_i^k) = 1/(exp(-\beta(x - \theta_i^k) + 1)$$

$$\frac{d\theta_i^k}{dt} = \epsilon(\Theta - \theta_i^k - \alpha p_i^k)$$

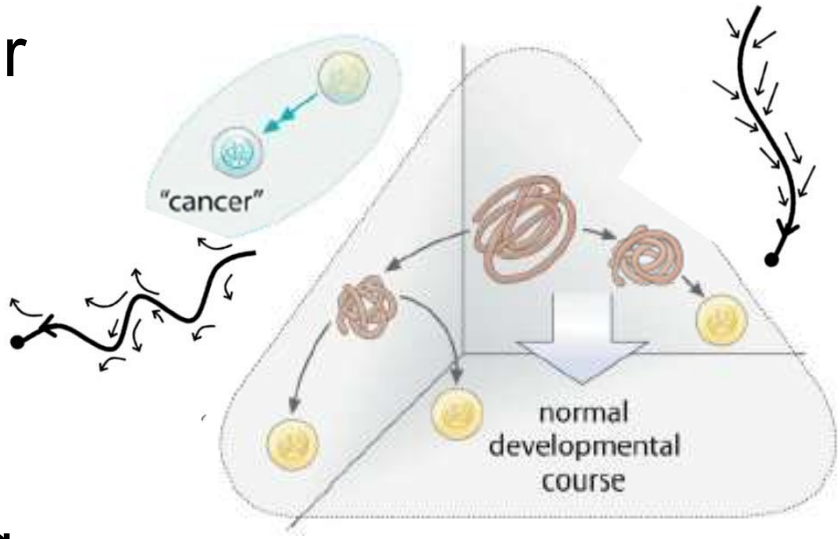
ε feedback rate

Θ, α, constant parameter

Speculation on Cancer State(?):

(KK,Bioessays2011)

(1)Through evolution, robustness to noise leads to robustness to mutation for normal cells



(2)When the GRN is complex there will appear 'aberrant' attracting states (depending on cell-cell interaction)

(3)(i)These states do not form stabilizing relationship with other cells ('selfish')

(ii) Since they are not an object for selection in evolution, they are generally not robust to mutation

(iii) With mutations, more stable states could be generated, thus mutations can be accumulated

(iv) Due to lack of robustness to noise, the phenotypes will be heterogeneous (not in time but over cells)

Cancer: interaction-dependence + loss of mutational robustness